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RESOLUTION AND COMPARATIVE ANTI-HIV EVALUATION OF THE ENANTIOMERS OF CALANOLIDES A AND B[1]

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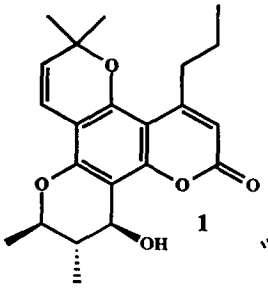
ABSTRACT: Methods for the chiral resolution of (+)-calanolide A and (-)-calanolide A from synthetic (±)-calanolide A, and of (+)-calanolide B and (-)-calanolide B (costatolide) from a scalemic mixture isolated from *C. lanigerium*, have been developed. Calanolide A was originally isolated as the pure (+) enantiomer from *C. lanigerum*, but now is also shown to occur as a scalemic mixture in latex of *C. teysmannii*. Interestingly, (+)-calanolide A and (-)-calanolide B are potent HIV-1 inhibitors, while (-)-calanolide A and (+)-calanolide B are inactive against the virus.

The novel coumarin derivative (+)-calanolide A (**1**) was first isolated and identified from leaves and twigs of the Malaysian rainforest tree *Calophyllum lanigerum* var. *austrocoriaceum* by our laboratory.[2] Its HIV-inhibitory activity was subsequently characterized as representative of a new class of non-nucleoside HIV-1 specific reverse transcriptase inhibitors.[3] As such, calanolide A has been selected by the NCI Division of Cancer Treatment's Decision Network Committee for preclinical drug development.

As with many natural products,[4] the procurement of adequate supplies of (+)-calanolide A for detailed biological evaluation has proved to be quite challenging. The compound is a minor component of the leaves ($\leq$1 mg/g extract) and is even less concentrated or completely undetectable in the more abundant bark and latex extracts.[5] Recently, scientists at SmithKline Beecham reported a synthesis of racemic calanolide A.[6] Since a chiral synthesis was not yet available, and because a comparison of the bioactivity of (+)-calanolide A and (-)-calanolide A was of interest, we initially pursued a chiral separation approach with synthetic (±)-calanolide A. Herein we report a simple and efficient semi-preparative separation of the enantiomers of calanolide A and their preliminary biological evaluation. We also describe the results of extension of this method to the resolution and biological evaluation of the enantiomers of the related[2] calanolide B.



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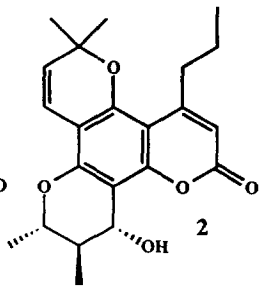

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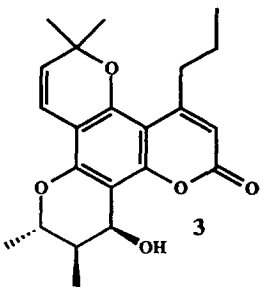

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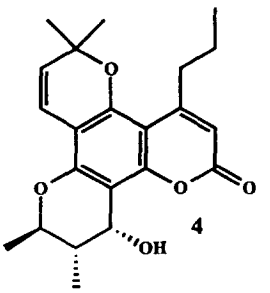

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[#]Program Resources, Inc./DynCorp, NCI-Frederick Cancer Research & Development Center, Frederick, MD

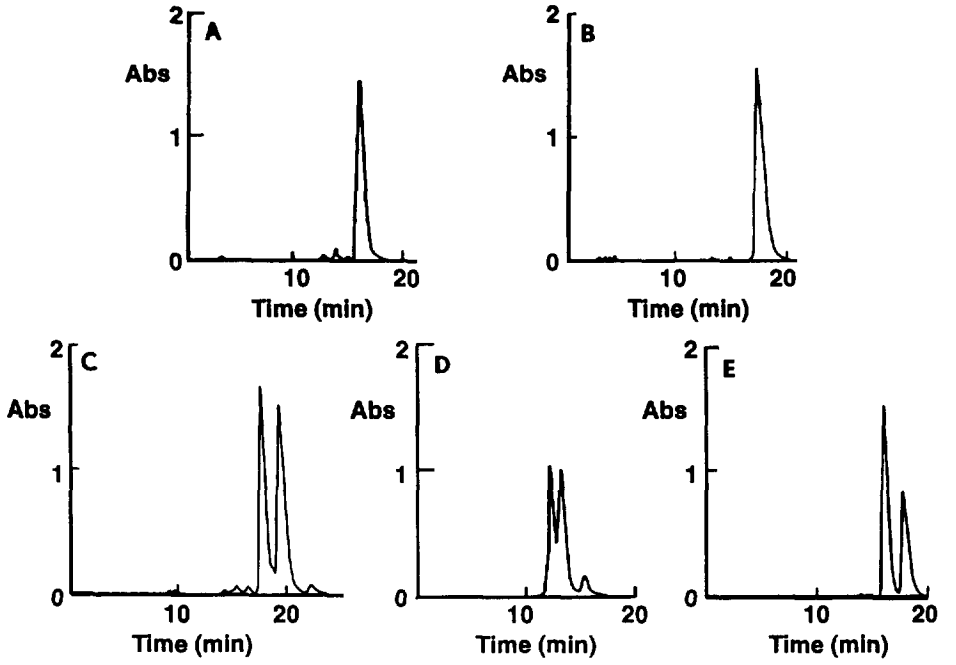

Figure 1. Chiral HPLC analyses of calanolide A, using a Waters 600 solvent delivery system and a Waters 990 diode array detector. Analytical runs (panels A-C, E) utilized an L-phenylglycine column (Rex-Chrom Pirkle, 4.6 x 250 mm), eluting with hexane-iPrOH (47:3) at 1 mL/min, with UV detection at 260 or 270 nm. Semi-preparative runs (panel D) utilized a D-phenylglycine column (ES Industries, 1 x 25cm), eluting with hexane-iPrOH (19:1) at 5 mL/min, with UV detection at 260 nm. A) natural (+)-calanolide A; B) (-)-calanolide A; C) synthetic, racemic calanolide A (SmithKline Beecham); D) synthetic, racemic calanolide A (Ash Stevens); E) calanolide A from latex of *C. teysmannii* var. *inophylloide*.

Natural (+)-calanolide A (**1**) was isolated from leaf extracts of *Calophyllum lanigerum* var. *austrocoriaceaum* as previously described;[2] (-)-calanolide A (**2**) was prepared in two steps (oxidation/reduction) from natural (-)- calanolide B (**3**).[5,7,8] The latter compound, also known as costatolide,[8] was first isolated by Stout and Stevens from *C. costatum*.[9] A reference sample of racemic, synthetic calanolide A was generously provided by SmithKline Beecham, while bulk quantities (0.3-1.0g) were provided by Ash Stevens and Program Resources, Inc., under contract to the National Cancer Institute.

Initial experiments with phenylglycine bonded phase columns revealed $\geq$90% resolution of (±)-calanolide A, but some drift in the retention times (Figure 1). Resolution of the synthetic racemates was further complicated by overlapping peaks from trace amounts of the calanolide B side products. Attempts to scale up the separation on a 1 x 25 cm L-phenylglycine column failed to achieve adequate resolution (Figures 1C, 1D).

When we set out to establish conditions for the separation of (-)-calanolide B (**3**)[5] and (+)-calanolide B (**4**),[2] (-)-calanolide B gave a single peak in chiral HPLC analyses (Figure 2A), but we were surprised to observe two chromatographic peaks from chromatographically (HPLC, TLC) and spectroscopically (NMR) "pure" natural (+)-calanolide B (Figure 2B). The L-phenylglycine bonded phase was amenable to semi-

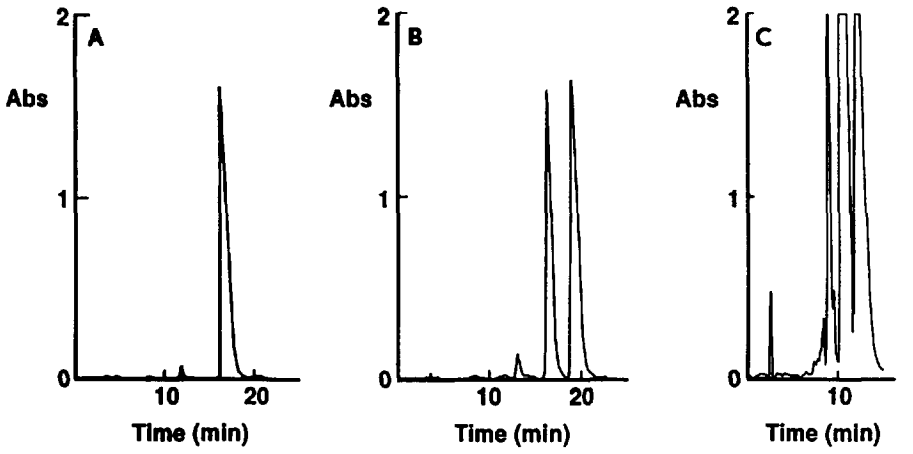

Figure 2. Analytical chiral HPLC separation of (+)-calanolide B and (-)-calanolide B (costatolide); conditions as described in Figure 1. A) (-)-calanolide B; B) natural "calanolide B"; C) semi-preparative resolution of natural "calanolide B" (5 mg injection).

preparative separation of the enantiomers **3** and **4**, yielding optically pure (+) and (-) calanolide B in an 11:9 ratio (Figure 2C). This ratio explains the observed optical rotation of (+)-calanolide B, $[\alpha]_D+10°$,[2] the calculated rotation from the enantiomeric excess of **4** in the mixture.

To resolve the enantiomers of calanolide A, we resorted to the Whelk O-1 column, which gave excellent resolution of the racemate **1/2** (Figure 3A). This separation was readily scaled up to 5 mg injections on a semi-preparative column (Figures 3B and 3C). While this work was ongoing, we isolated what appeared to be calanolide A (by chromatographic retention time and NMR analysis) as a minor (<0.1%) component of the latex extract from *Calophyllum teysamnnii* var. *inophylloide*, but the observed optical rotation of this material was low, $[\alpha]_D+15.1°$ vs. $[\alpha]_D+60°$.[2] Chiral analysis revealed that the calanolide A fraction from this latex was a scalemic mixture of **1** and **2**, with an enantiomeric excess of (+)-calanolide A (Figure 1E).

Preliminary comparative evaluation of the anti-HIV activity of the four purified enantiomers revealed that natural (+)-calanolide A and (-)-calanolide B (costatolide) were potent inhibitors of HIV-1 *in vitro* (EC_{50} 0.2 and 0.3 μM, respectively), but that (-)-calanolide A and (+)-calanolide B were devoid of this antiviral activity. Therefore, the anti-HIV activity originally observed and reported[2] for "calanolide B" was due to the presence of (-)-calanolide B (costatolide, ~45%) in the scalemic mixture comprising the tested sample.

These results further clarify the stereochemical assignments in this class of compounds and illustrate further that very subtle changes in stereochemistry and conformation may lead to profound differences in biological activity.

ACKNOWLEDGEMENT: We thank SmithKline Beecham, the staff of the NCI Drug Synthesis and Chemistry and Natural Products Branches and their contractors, Ash Stevens and Program Resources, Inc., for samples of synthetic racemic calanolide A. We also thank Louis Glunz (Regis) for the loan of an analytical Whelk O-1 column, and Dr. J.B. McMahon and R. Gulakowski for anti-HIV assays.

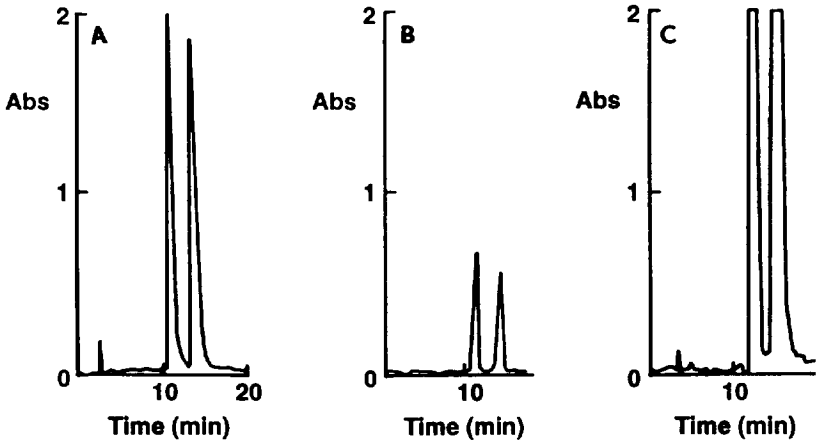

Figure 3. Chiral HPLC resolution of synthetic, racemic calanolide A, using HPLC system described in Figure 1. Analytical runs (panel A) employed an S,S Whelk O-1 column (Regis, 4.6 x 250 mm), eluting with hexane-iPrOH (9:1) at 1.25 mL/min, with UV detection at 270 nm. Semi-preparative runs utilized an S,S Whelk O-1 column (Regis, 1 x 25 cm), eluting with hexane-iPrOH at 5 mL/min, with UV detection at 270 nm. A) (+)-calanolide A analytical (0.10 mg), B) semi-preparative (0.5 mg); C) semi-preparative (5 mg).

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8. Despite the fact that the name "costatolide" has chronological precedent, we strongly urge that compound **3** henceforth be called (-)-calanolide B for the following reasons: a) the structure originally presented[9] for "costatolide" is actually (+)-calanolide B; b) the name "costatolide" was subsequently used for an unrelated marine natural product;[10] and c) the term "calanolide class" of non-nucleoside reverse transcriptase inhibitors has appeared several times in the literature[3,11] and will likely continue to be used.
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