

ANTI-HIV COUMARINS FROM CALOPHYLLUM SEED OIL

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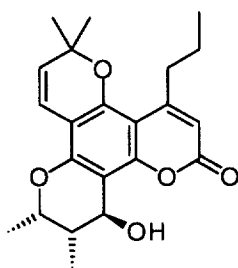
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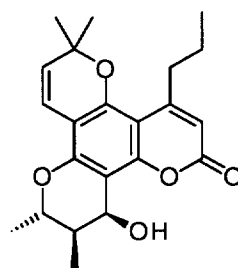
Abstract: The seeds of *Calophyllum cerasiferum* Vesque (Family - Clusiaceae), and *Calophyllum inophyllum* Linn. (Family - Clusiaceae) contain several known coumarins, among which were the potent HIV reverse transcriptase inhibitors costatolide and inophyllum P. *Calophyllum cerasiferum* contained (-)-calanolide B as its major coumarin constituent in significant amount and thus constitute a renewable source of this compound.

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Calanolide A (**1**) and costatolide (**2**) belong to a new class of HIV-1 reverse transcriptase inhibitors.^{1,2} The former has been designated for preclinical trials by the U.S. National Cancer Institute, while its isomeric analogue, **2**, is retained as an alternative for drug development.³ Other pyranocoumarins such as soulattrolide **4** isolated from the latex of certain *Calophyllum* species were found to be active against HIV.³ Acquiring sufficient quantities of **1** for preclinical and clinical development presented a colossal problem since only minute amounts (<1 mg/g extract) of calanolide A could be isolated from the leaves of *Calophyllum lanigerum* var. *austrocoriaceum*.⁴ Since this original source of (+)-calanolide A was destroyed,⁵ finding new sources of active pyranocoumarins has become a worthwhile endeavour. In 1994, researchers at NCI reported on the isolation of pyranocoumarins with known anti-HIV activity from the latex of the tropical rainforest tree *Calophyllum teysmannii* var. *inophylloide*.³ Harvesting the latex, a renewable source, can be non destructive for the trees and thus presented hope of getting adequate supply of costatolide. Costatolide represented 48% of the extractable from the latex, but the approximate weight of extractable per unit weight of latex was not given. Recently, total syntheses of optically active calanolide A and B have appeared in the literature.⁶



(+)-Calanolide A **1**



(-)-Calanolide B **2** (Costatolide)

We surveyed the anti-HIV activity of a number of extracts from the seeds of several *Calophyllum*, and other, species acquired in Fiji and surrounding Polynesian isles. Two crude extracts (hexanes) displayed marked anticytophatic activity against HIV-1 IIIB strain from 10 $\mu\text{g/mL}$ concentration in C8166 cells. One of the samples, namely a seed extract from *Calophyllum cerasiferum* Vesque (Family - Clusiaceae)⁷ contained (-)-calanolide B (costatolide). NMR, mass spectrometry, and optical rotation confirmed the structure of the isolated compound. Over 7 mg/g of extract were obtained and purification was straightforward using normal flash-chromatography on a silica gel column (partition between hexanes and methanol to remove triglycerides and other lipids, then chromatography using hexanes/10% EtOAc). This means that 1 Kg of crushed seeds, which yielded 55 g of crude extract, yields nearly 0.4 g of pure costatolide.

Figure 1 gives an example of the anticytophatic activity of the crude extract from *Calophyllum cerasiferum* containing costatolide. Small amounts of two unidentified coumarins were also present in this extract. They could be easily separated from costatolide but were isolated as a mixture difficult to analyse. They are thought to belong to the inophyllolide family as judged from the NMR and mass spectra.

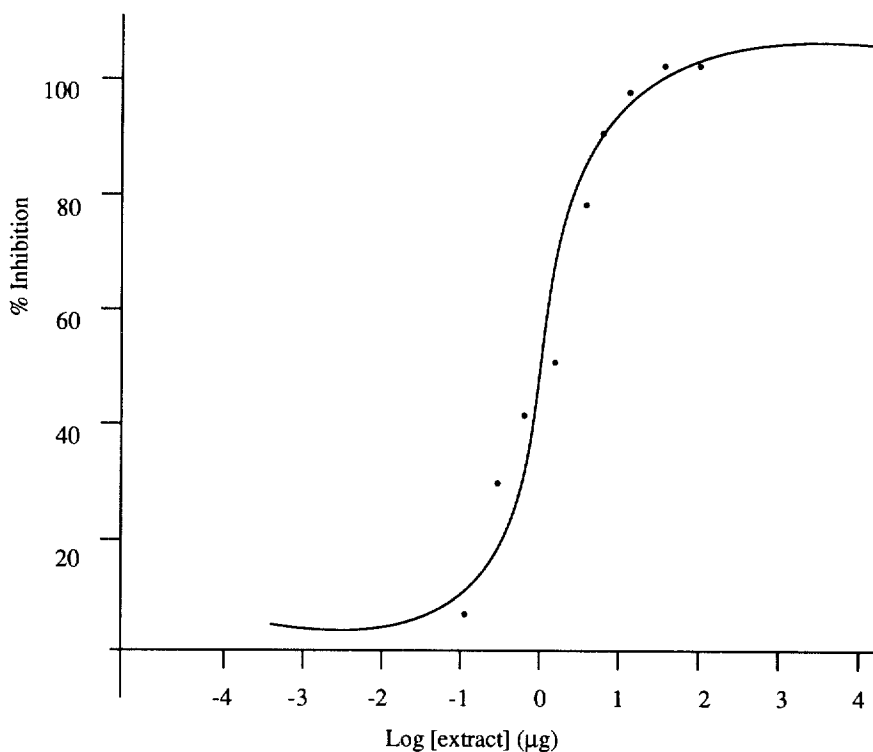
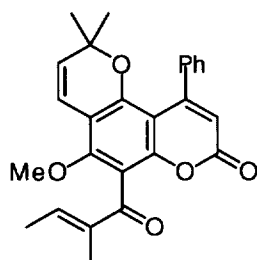
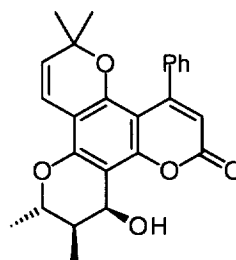
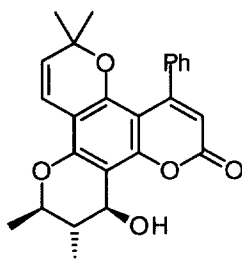


Figure 1. Anti-HIV activity of crude extract of *Calophyllum cerasiferum* in an ELISA-based in vitro assay (HIV-1 p24 antigen) of cytopathicity of HIV-1 IIIB strain to C8166 cells.

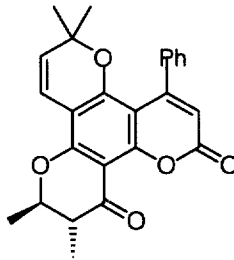
The other active sample was shown to contain more pyranocoumarins than the previous one. The sample originated from *Calophyllum inophyllum* Linn. (Family - Clusiaceae).⁸ Calophyllolide **3** was the major compound and several inophyllolides including inophyllum B (**5**), C (**6**), P (**4**), and E (**7**) were identified. We presume that the anti-HIV activity was due to the presence of soullatrolide (inophyllum P),^{3,9} although two more related compounds could be seen but were found in such small quantities that their identification has not been possible so far. Calophyllolide, inophyllum C and E could be freed from other coumarins (90-95% purity) while inophyllolides B and P were isolated as a mixture and identified from their respective proton NMR signals.



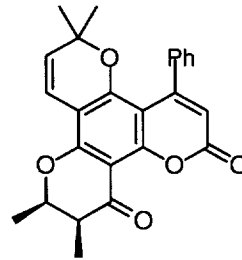
3 Calophyllolide

4 Inophyllum P
(Soullatrolide)

5 Inophyllum B



6 Inophyllum C



7 Inophyllum E

The finding that *Calophyllum* seed oil contains HIV reverse transcriptase inhibitors, especially costatolide, represent a significant progression in the search for renewable sources of these pyranocoumarins. Harvesting the seeds is non destructive for these plants that grow abundantly in Fiji Islands. Seed oil from *Calophyllum inophyllum* has been used in folk medicine to treat aching joints and rheumatism.⁸ Although, we have found that this extract was cytotoxic, that from *Calophyllum cerasiferum* was not at the highest concentration tested (50 µg/mL).

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