New Pyranocoumarins Isolated from Calophyllum lanigerum and Calophyllum teysmannii

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HIV-INHIBITORY COUMARINS FROM LATEX OF THE TROPICAL RAINFOREST TREE *Calophyllum teysmannii* var. *inophylloide*[1]

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ABSTRACT: In an effort to identify an adequate and sustainable natural source of the recently described anti-HIV drug development candidate calanolide A, we undertook chemical and biological studies of the latex exuded from trees of the genus *Calophyllum*. Although we found that calanolide A was not present in latex from the original source species, *C. lanigerum* var. *austrocoriaceum*, we did observe that a related coumarin, costatolide, was abundant in latex of *C. teysmannii* var. *inophylloide*. Costatolide is currently being evaluated as a possible alternative to calanolide A for drug development.

Calanolides A (**1**) and B (**2**) are among a series of coumarins recently reported[2] from *Calophyllum lanigerum* Miq. var. *austrocoriaceum* (C. T. Whitmore) Stevens from the rainforest of Sarawak, Malaysia. Both **1** and **2** were strongly inhibitory against the *in vitro* replication and cytopathicity of the human immunodeficiency virus type 1 (HIV-1). Further investigation has revealed that calanolide A represents a novel subset among the more general class of HIV-1 specific reverse transcriptase inhibitors.[3-5] On the basis of these findings, calanolide A has been committed to preclinical drug development by the U. S. National Cancer Institute.

The amounts of natural calanolide A that would be required for preclinical development, and possibly clinical trials and clinical development thereafter, present a formidable isolation challenge. The best yields obtained from the original collection were ~1 mg/g extract (of leaves), meaning that quite large collections of leaves, perhaps tons, would be required. When preliminary surveys revealed that the source, *C. lanigerum* var. *austrocoriaceum*, was not particularly abundant, an alternative strategy emerged. A review of the literature indicated that other coumarins had been obtained in high yield from the latex of certain *Calophyllum* species;[6] this suggested an attractive option, provided such compounds could also be shown to have

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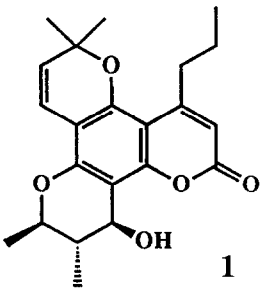
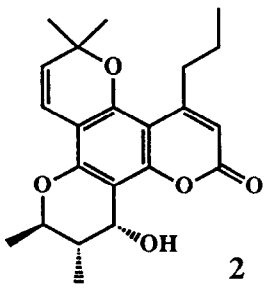
anti-HIV activity similar to calanolide A. Latex collections could easily be made by making several small slash wounds, about 2 cm wide, in the bark of mature trees. Exuding latex would collect in these cuts and could then be harvested by scraping. These latex harvests could, in theory, be sustained over an extended period of time and would be non-destructive; the wounds healed after a period of time and new slashes could be made. The trees would not die from such treatment and could be used repeatedly for such collections.

We were disappointed to find that the latex from numerous specimens of *C. lanigerum* var. *austrocoriaceum* contained no discernible amounts of calanolide A, nor was the latex active in our anti-HIV assay.[7] By far the major component of the latex was the ring-opened ketone **3**, which we had found earlier in leaves and twigs of the same tree.[2] We then began to survey latex collections of various species of *Calophyllum* from Sarawak for the presence of the calanolides. Latex from a tree subsequently identified as *C. teysmannii* Miq. var. *inophylloide* (King) Stevens (voucher specimen Soejarto & Jude 7605) was active against HIV,[7] and appeared (TLC, NMR) to contain significant amounts of calanolide B.



1



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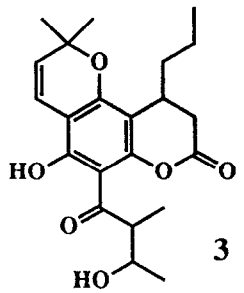

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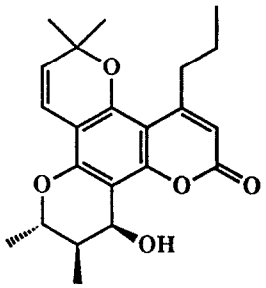

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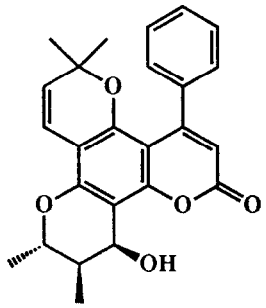

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Preparative separation of the putative calanolides from the latex by extraction (warm CH_2Cl_2-MeOH, 1:1), vacuum liquid chromatography (silica, EtOAc) and HPLC (silica, hexane-EtOAc, 7:3) provided a compound identical to calanolide B (48% of the latex extractables[8]) by NMR and mass spectrometry, and soulattrolide[9] (**5**, 29% of latex extractables), in a ratio of ~ 3:2. The structures were verified by comparison with literature data. Optical rotation measurements, however, revealed that the former compound was, in fact, costatolide

(4),[6] the enantiomer of calanolide B. Both 4[6] and 5[9] were previously known natural products; however, they were not previously known to have antiviral activity. Compounds related to soulattrolide, the inophyllums, were very recently reported as inhibitors of HIV-1 reverse transcriptase by the research team at Smith Kline Beecham.[10]

Soulattrolide was similar in potency to calanolide B in preliminary comparative anti-HIV tests,[7] while costatolide, 4, was intermediate in potency between calanolides A and B. Figure 1 gives an example of the anticytopathic activity of costatolide against HIV-1 in CEM-SS cells using the XTT tetrazolium assay.[7] Continuing field surveys have identified more than a dozen trees from which latex containing consistently high levels of 4 could be repeatedly harvested. Based upon its substantial anti-HIV activity and potentially more ready access to larger quantities by sustained harvesting of latex, costatolide is being explored as a possible alternative development candidate to calanolide A.

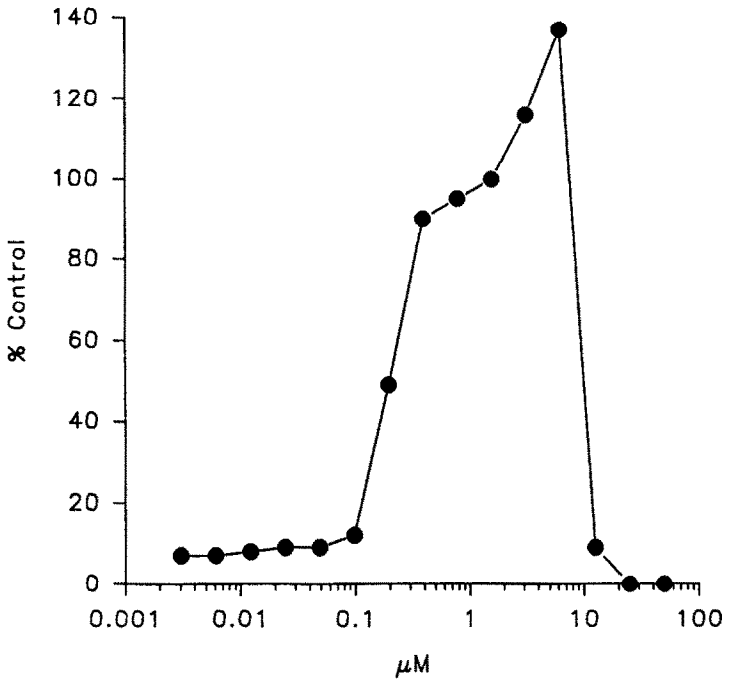

Figure 1. Example of anti-HIV activity of costatolide in an XTT-based *in vitro* assay of cytopathicity of HIV-1 to CEM-SS cells.

Acknowledgements

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References and Notes

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