# Pyranocoumarins from Tropical Species of the Genus *Calophyllum*: A Chemotaxonomic Study of Extracts in the National Cancer Institute Collection[1]

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(+)-Calanolide A, a novel dipyranocoumarin from the Malesian tree *Calophyllum lanigerum* var. *austrocoriaceum*, and a closely related compound, (−)-calanolide B, isolated from *Calophyllum teysmannii* var. *inophylloide*, are representatives of a distinct class of nonnucleoside HIV-1 specific reverse-transcriptase inhibitor under development as an AIDS chemotherapeutic. NCI repository specimens totalling 315 organic extracts from 31 taxa of *Calophyllum* were analyzed for related pyranocoumarins using a simple TLC system. A total of 127 extracts was initially classified as "positive"; eight out of the 31 taxa examined, representing perhaps 28 species already described ($^1/_7$−$^1/_8$ of all the species in this genus), contained prenylated coumarins, suggesting that these compounds, while sometimes abundantly present, are not widespread in the genus. Representative members of the TLC-positive extracts were partitioned between $CH_2Cl_2$ and 25% aqueous MeOH; the $CH_2Cl_2$-soluble materials were then analyzed by TLC and $^1$H NMR to confirm the presence of pyranocoumarins. The anti-HIV activity of the partitioned extracts are also presented. This study suggested that there are several distinctive coumarin chemotaxonomic markers distinguishing species of this genus.

Plants from the genus *Calophyllum* (Guttiferae, Clusiaceae) have proven to be a rich source of natural products, including xanthones,[2−4] steroids,[5] triterpenes,[6,7] coumarins,[8−10] and benzopyrans.[11] In 1992, we reported a series of dipyranocoumarins, the calanolides, from *C. lanigerum* var. *austrocoriaceum*.[12] (+)-Calanolide A (**1**) is the prototype of a unique subclass[13−15] within the general class comprising nonnucleoside HIV-1 reverse transcriptase (RT) inhibitors.[16] The compound is fully active against strains of HIV-1, which are resistant to diverse other nonnucleoside as well as nucleoside (e.g., AZT) RT inhibitors.[15] The first in vivo studies of (+)-calanolide A in humans began in June 1997.[17]

Numerous pyranocoumarins have been isolated from various species of *Calophyllum*. These compounds fall into three basic structural groups: (a) tetracyclic dipyranocoumarins in which the C rings have a *gem*-dimethyl group; [examples include (+)-calanolide A (**1**),[12] (−)-calanolide B (**2**),[18,19] the inophyllums (e.g., **3**),[20−23] and the cordatolides (e.g., **4**)[24]]; (b) tetracyclic dipyranocoumarins with reversed C and D pyran rings [i.e., the *gem*-dimethyl groups are found in the D ring, as in the pseudocalanolides (**5**, **6**)[25]]; and (c) tricyclic pyranocoumarins (e.g., **7**),[12] which contain a noncyclized equivalent of the D ring of the calanolide structure class. Individual members of these three structural groups also vary with respect to the C-4 substituent on the lactone ring of the coumarin, where methyl, *n*-propyl, or phenyl groups have been encountered.

A critical requirement for any natural product under consideration as a drug-development candidate is an adequate supply of the compound for preclinical, and possibly clinical, development. In the case in which the plant source of the lead compound is scarce or very rare, one approach to resolving the supply problem is to attempt to identify other, more abundant natural sources of the compound in question. With this in mind, we undertook a chemotaxonomic study of the *Calophyllum* extracts present in the NCI Repository in an effort to identify alternative sources of the calanolides or related compounds with similar bioactivity.

## Results and Discussion

The genus *Calophyllum* is a large group of tropical trees consisting of approximately 180−200 different species. Although a handful of species has been identified in the New World, the genus is primarily found in the Indo-Pacific region, particularly Malesia.[26,27] As of January 1996, the NCI Repository contained 315 organic extracts of plants from the genus *Calophyllum*, which had been collected under contract. The NCI collection contained samples from 31 different taxa—*C. biflorum*, *C. blancoi*, *C. brasiliensis*, *C. canum*, *C. castaneum*, aff. *castaneum*, *C.* cf. *depressinervosum*, *C. ferrugineum* var. *occidentale*, *C. glaucescens*, *C.* cf. *incumbens*, *C. inophyllum*, *C. lanigerum* var. *austrocoriaceum*, *C. mariae*, *C. molle*, *C. nodosum*, *C.* cf. *nodosum*, *C. obscurum*, *C. papuanum*, *C. parviflorum*, *C. pauciflorum*, *C.* aff. *pervillei*, *C. sclerophyllum*, *C. soulattri*, *C. sundaicum*, *C.* cf. *sundaicum*, *C. tacamahaca*, *C. tetrapterum*, *C. teysmannii* var. *inophylloide*, *C. teysmannii* var. *teysmannii*, *C. venulosum*, *C. wallichianum* cf. var. *incrassatum*, and *C. woodii*—and 32 extracts (from 10

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**Table 1.** HIV Testing Results of Representative Coumarin Positive *Calophyllum* Extracts

| species | NSC # | voucher specimen[a] | plant part[b] | $CH_2Cl_2$ ($EC_{50}$ μg/mL) | $CH_2Cl_2$ ($IC_{50}$ μg/mL) | extract ($EC_{50}$ μg/mL) | extract ($IC_{50}$ μg/mL) |
|---|---|---|---|---|---|---|---|
| *C.* aff. *biflorum* Henderson &Wyatt-Smith | N5523 | Me 122592 | BR | 13 | 20 | 40%8[c] | 28 |
| | N26825 | Me 122592 | LF | NP[d] | 36 | NP[d] | 46 |
| *C. blancoi* Planchon & Triana | N25311 | SMa 6802 | LF/TW | NP[d] | 6 | NP[d] | 13 |
| | N26021 | SMa 6802 | RT | 4 | 6 | 40%8[c] | 14 |
| | N26053 | SMa 6802 | SB | NP[d] | 25 | NP[d] | 43 |
| *C.* aff. *castaneum* PF Stevens | N43519 | Fr 2200 | LF | NP[d] | 25 | NP[d] | 35 |
| *C. inophyllum* L. | J2207 | PM 4476 | WD | 3 | 13 | 8 | 26 |
| | N19671 | F 788 | TW | 10 | 28 | NP[d] | 24 |
| | N19837 | F 788 | LF | 30%16[c] | 15 | NP[d] | 61 |
| | N37901 | M 4260 | LF | 40%8[c] | 9 | 40%4[c] | 5 |
| | N181122 | PC 0028 | PL | 7 | 23 | NP[d] | 154 |
| | N49953 | SF 7649 | LF/TW | NP[d] | 80 | NP[d] | 9 |
| | N49965 | SFS 7774 | LF/TW | NP[d] | 14 | NP[d] | 38 |
| | N67211 | MS 44013 | FR | 24 | 84 | 9 | 49 |
| *C. lanigerum* Miq. var. *austrocoriaceum* | N10865 | BL 351 | FR/TW | <2 | 25 | 4 | 81 |
| (TC Whitmore) PF Stevens | N10907 | BL 351 | LF | 5 | 55 | NP[d] | 86 |
| | N45069 | S 7861 | TW | 40%32[c] | 30 | 40%32[c] | 24 |
| | N45071 | S 7863 | TW | 15 | 36 | 26 | 42 |
| | N45073 | S 7867 | TW | 14 | 24 | 16 | 32 |
| | N45273 | SOJ 7872 | LF | 2 | 15 | 12 | 58 |
| | N45275 | SOJ 7872 | TW | 5 | 22 | 11 | 42 |
| | N46637 | S 7867 | LF | 40%8[c] | 12 | 35%32[c] | 49 |
| | N46639 | S 7862 | LF | 45%16[c] | 19 | 35%32[c] | 50 |
| *C. lanigerum* Miq. var. *austrocoriaceum* | N46641 | S 7863 | LF | 58 | 107 | 29 | 42 |
| *C. mariae* Tr. & Pl. | N30659 | D 3243 | BK | NP[d] | 61 | NP[d] | 10 |
| | N31011 | D 3243 | WD | NP[d] | 108 | NP[d] | 17 |
| *C. molle* King | N19563 | B 1290 | LF | 30%32[c] | 42 | NP[d] | 88 |
| | N19607 | B 1290 | BK | NP[d] | 25 | NP[d] | 40 |
| | N43339 | SS 264 | LF/TW | 16 | 22 | 40%16[c] | 38 |
| | N43515 | SS 264 | SB | NP[d] | 41 | NP[d] | 48 |
| *C. nodosum* Vesque | N43343 | Me 109841 | LF | NP[d] | 24 | 30%64[c] | 69 |
| | N43513 | Me 109841 | FL | NP[d] | 36 | 40%32[c] | 5 |
| *C.* cf. *nodosum* Vesque | N5567 | Me 122594 | BR | 31 | 37 | 32 | 43 |
| | N22969 | Me 122594 | LF | 45%32[c] | 41 | NP[d] | 83 |
| | N64665 | MM 131987 | BR | 45%16[c] | 17 | 45%16[c] | 27 |
| | N64793 | MM131987 | LF | 40%8[c] | 18 | 20 | 56 |
| *C. papuanum* Lauterb. | N43347 | TS 6998 | FR/LF/TW | NP[d] | 38 | NP[d] | 38 |
| *C.* aff. *pervillei* Drake | N39171 | Z 7595 | ST/TW | NP[d] | 41 | NP[d] | 38 |
| | N39187 | Z 7595 | TW | 8 | 10 | 30%8[c] | 22 |
| | N39205 | Z 7595 | LF | 4 | 7 | 40%16[c] | 18 |
| *C. soulattri* Burman f. | N22859 | SRSE 6607 | FR | NP[d] | 2 | NP[d] | 2 |
| | N23213 | SRSE 6607 | SB | TX[e] | <2 | NP[d] | 3 |
| | N25863 | SRSE 7056 | RT | TX[e] | <2 | TX[e] | <2 |
| | N45265 | SOJ 7871 | FR | NP[d] | 21 | NP[d] | 20 |
| | N49973 | SFR 7738 | SB | NP[d] | <2 | NP[d] | 2 |
| | N68493 | MI 3890 | LF | NP[d] | 32 | 20%32[c] | 29 |
| | N68193 | MI 3890 | BK | NP[d] | <2 | NP[d] | 2 |
| *C. tacamahaca* Willd. | N49951 | L 4538 | LF | 2 | 8 | 2 | 12 |
| *C. teysmannii* Miq var. *inophylloide* | N38047 | SM 7605 | BR | <2 | 3 | <2 | 26 |
| (King) PF Stevens | N38055 | SM 7605 | LF/TW | <2 | 21 | <2 | 22 |
| | N46481 | S 7899 | SB | <2 | 48 | <2 | 22 |
| | N46483 | S 7854 | LF | 4 | 22 | 5 | 36 |
| | N46485 | S 7854 | TW | <2 | 11 | 3 | 57 |
| | N46489 | S 7902 | SDL | <2 | 16 | 6 | 91 |
| | N46491 | K 1355 | SD | <2 | 14 | 10 | 95 |
| | N63987 | MM 131885 | LF | 30%8[c] | 10 | 40%64[c] | 61 |
| | N64011 | MM 131885 | BR | NP[d] | 17 | 40%16[c] | 19 |
| *C. wallichianum* Pl. & | N64783 | MM 131984 | LF | NP[d] | 49 | NP[d] | 34 |
| Tr. cf. var. *incrassatum* (Henderson & Wyatt-Smith) PF Stevens | N64669 | MM 131984 | BR | NP[d] | 4 | 40%32[c] | 41 |

[a] One set of voucher specimens has been deposited at the herbarium of a host institution in the country of collection, while a second set is preserved at the U.S. National Herbarium or the Smithsonian Institution, Washington, DC. Collector name codes: B = Burley et al., BL = Burley & Lee, D=Devia, F = Fernando, Fr = Frodin, K = Kadushin, DD Soejarto & Ismawi, L = Lowry, M = Miller, Me = Meijer, MI = McDonald & Ismail, MM = Meijer & Madani, MS = Mandlik & Shigvan, PC = P Cox, PM = P. Murphy, S = Soejarto et al., SF = Soejarto & Fernando, SFR = Soejarto, Fernando & Reynoso, SFS = Soejarto, Fernando & Sagcal, SMa = Soejarto & Majaducon, SM = Soejarto & Mohtar, SOJ = Soejarto, Othman, & Jude, SRSE = Soejarto, Reynoso, Sagcal, & Edrada, SS = Soepadmo & Suhaimi, ST = Stijfhoorn, T = Takeuchi, TS = Takeuchi & Soejarto, Z = Zarucchi, Rakatobe, Razafimandimbson, & Pool. [b] Two-letter code for plant parts are: BK = bark, BR = branch, FL = flower, FR = fruit, LF = leaf, PL = plant, RT = root, SB = stembark, SD = seed or SDL = seedling, ST = stem, TW = twig, WD = wood. [c] Test samples that did not reach 50% protection were described by the highest level of protection reached and the dose at which the level was reached (eg., 40%8). [d] NP, no protection from HIV-induced cell killing. [e] TX, toxic at lowest dose tested.

trees), which were identified only to the genus level. The extracts were obtained from 121 different trees. Multiple extracts from different plant parts were often taken from a single tree to facilitate identification of the part(s) richest

in any metabolites of interest. The largest groups of extracts were from trees of the species *C. inophyllum* (8 trees), *C. lanigerum* (13 trees), *C. soulattri* (11 trees), and *C. teysmannii* (30 trees, total from both varieties of *C. teysmannii* collected).

In the initial phase of this study, we utilized TLC to identify those extracts that contained coumarins. A small aliquot of the crude extract was dissolved in $CH_2Cl_2$ and chromatographed against (−)-calanolide B (**2**) and soulattrolide (**8**), representing the 4-*n*-propyl and 4-phenyl subclasses, on Si gel plates. The plates were developed with hexane−EtOAc (7:3), allowed to air-dry, and then sprayed with vanillin−$H_2SO_4$; over the course of several hours, pyranocoumarin spots turned a characteristic deep blue. In this analysis, 127 extracts showed TLC behavior consistent with the presence of pyranocoumarins. A few of the analyses were questionable, as color analysis was complicated by green pigments with similar $R_f$ values. Results from the TLC study indicated that many of the *Calophyllum* species in the NCI collection did not contain pyranocoumarins. These extracts, which were dropped from further consideration, were from the following species: *C. biflorum*, *C. brasiliensis*, *C. castaneum*, *C.* aff. *castaneum*, *C.* cf. *depressinervosum*, *C. ferrugineum* var. *occidentale*, *C. glaucescens*, *C. hosei*, *C. incumbens*, *C. parviflorum*, *C. sclerophyllum*, *C. sundaicum*, *C.* cf. *sundaicum*, *C. tetrapterum*, *C. wallichianum* cf. var. *incrassatum*, and *C. woodii*. The remaining pool of extracts provided somewhat ambiguous results; some extracts appeared to contain pyranocoumarins, while others, even from the same tree, did not.

Representative extracts for each species designated positive by this TLC procedure were analyzed more specifically for the presence of coumarins. A 100-mg aliquot of each crude organic extract was partitioned between 25% aqueous MeOH and $CH_2Cl_2$. The resulting fractions, along with the original crude extract, were tested[28] for anti-HIV activity; a $^1$H NMR spectrum of each $CH_2Cl_2$-soluble fraction was obtained. Finally, the resulting $CH_2Cl_2$ fractions were reassessed in the TLC system. These data are summarized in Table 1. Of the 127 extracts originally identified as positive for coumarins, 79 were partitioned and, of these, 47 were confirmed to contain such compounds. These extracts belonged to one of only eight species (*C. inophyllum*, *C. lanigerum* var. *austrocoriaceum*, *C. molle*, *C. nodosum*, aff. *pervillei*, *C. soulattri*, *C. tacamahaca*, or *C. teysmannii*) or to extracts that were identified only as *Calophyllum* sp.

We then undertook a detailed analysis of the coumarins present in several of the coumarin-positive extracts from *C. lanigerum*, *C.* aff. *pervillei*, *C. molle*, and *C. teysmannii*. Pyranocoumarins were isolated using a standardized scheme involving solvent−solvent partitioning, followed by vacuum-liquid chromatography on Si gel and finally HPLC [Si gel, hexane−EtOAc (7:3), occasionally followed by reverse-phase $C_{18}$, MeOH−$H_2O$ (9:1)]. The structures of the purified individual coumarins were then determined using conventional spectroscopic techniques.

Of the 25 extracts identified as *C. lanigerum* or *C. lanigerum* var. *austrocoriaceum*, 16 were identified as containing pyranocoumarins, including the two extracts from which the calanolides were originally isolated.[12] In-depth chemical analysis of the pyranocoumarins present in nine additional extracts led to the isolation of five compounds. The isolated coumarins included cordatolide A (**4**); tricyclic, or D-ring opened, calanolide E (**7**, originally isolated with the calanolides); one of its diastereomers, calanolide E2 (**9**); cordatolide E (**10**) with a methyl substituent at C-4; calanolide F (**11**), a new C-10 epimer of (+)-calanolide A; and pseudocordatolide C (**12**), the pyrano-ring reversed analogue with a C-4 methyl substituent. This was the first isolation of compounds **9**−**12**; details of their isolation and structure elucidation have been reported elsewhere.[29] Of the pyranocoumarins isolated from *C. lanigerum* extracts, the most commonly occurring were **7**, which was isolated from six extracts, and **12**, which was found in seven extracts. Together with previously described calanolides, this species of *Calophyllum* is now documented to produce 13 different pyranocoumarins representing all three structural types (see above), with either *n*-propyl or methyl substituents on the coumarin core. The extracts of *C. lanigerum* also contained multiple diastereomers of these pyranocoumarins (e.g., **1**, **2**, and **11**; **7** and **9**).

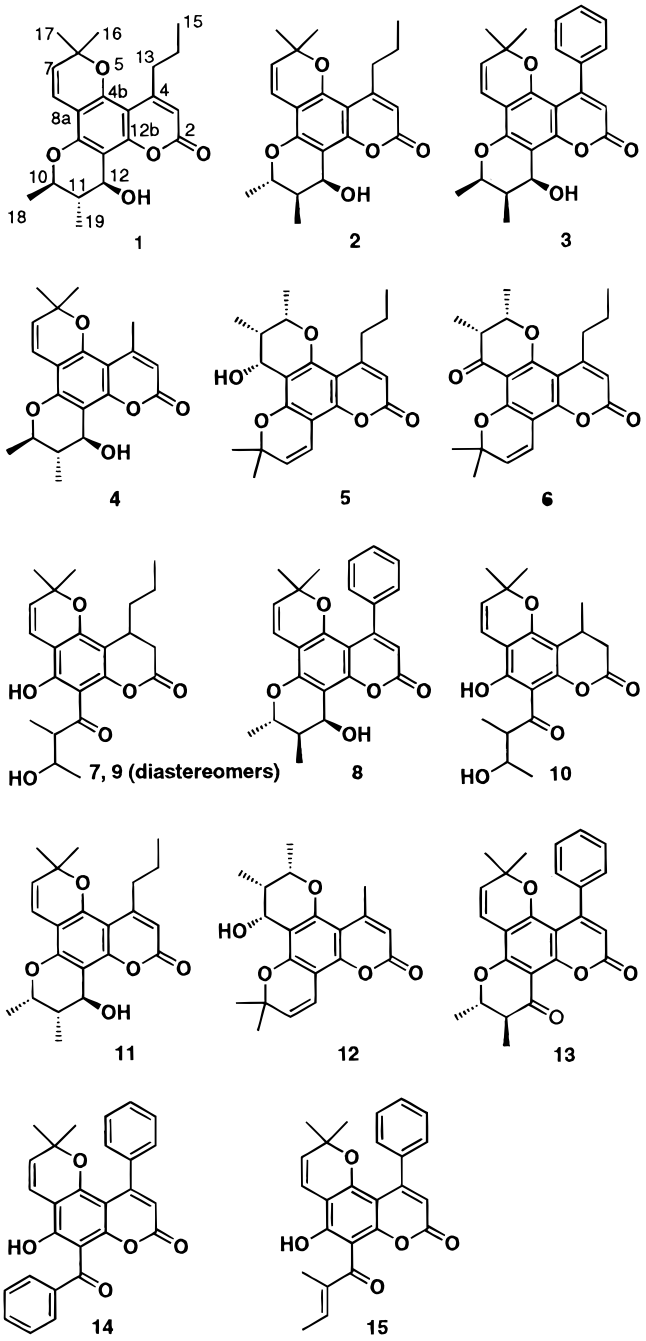
Thus far, *C. teysmannii* var. *inophylloide* has shown much less diversity in pyranocoumarin content, and only four such compounds have been found in the NCI collection. The two most abundant pyranocoumarins produced by this species were (−)-calanolide B (**2**) and soulattrolide (**8**), which differ only in the coumarin C-4 substituent (*n*-propyl vs phenyl, respectively). We have also isolated soulattrolone (**13**), the 12-keto derivative of soulattrolide (**8**), and a novel phenylketopyranocoumarin, calanone (**14**).[29] It is noteworthy that two chemotypes of *C. teysmannii* var. *inophylloide* seem to exist; one (Sampadi population) produces large amounts of **2** and **8**,[19] while the other (Semengoh population) contains **14** as the primary constituent[30] along with minor amounts of **13**.

Much like *C. teysmannii*, the collection of *C. molle* from Malaysia displayed very little diversity in its pyranocoumarin constituents. The only coumarin isolated from this collection was compound **7**, a tricyclic pyranocoumarin abundant in *C. lanigerum*. There was no sign of the diastereomer **9** or the methyl side-chain analogue **10**, which were present in *C. lanigerum*. Extracts of *C.* aff. *pervillei* from the NCI Repository also showed little variation in their pyranocoumarin metabolites. These extracts contained calophyllolide (**15**), a tricyclic pyranocoumarin first described in 1957 from *C. inophyllum*.[31,32]

We did not explore further the coumarin-containing extracts from *C. inophyllum* and *C. soulattri*, because the chemistry of these species had already been extensively explored by several groups. For example, soulattrolide (**8**) was first isolated and reported in 1977 from *C. soulattri* by Gunasekera and co-workers.[23] More recently, the pyranocoumarins from *C. inophyllum* were described by a group at SmithKline Beecham.[20] The latter report included several new pyranocoumarins, some of which were shown to inhibit HIV-1 RT.

As previously reported,[19] we have isolated (−)-calanolide B (**2**) from the latex of *C. teysmannii* var. *inophylloide*; this compound is considered a possible alternative to (+)-calanolide A for drug development. During the process of identifying *C. teysmannii* var. *inophylloide* latex as a renewable resource of (−)-calanolide B, the latex of more than 120 *Calophyllum* trees was examined for the presence of pyranocoumarins. The majority of the latex samples were obtained from either *C. lanigerum* var. *austrocoriaceum* or *C. teysmannii* var. *inophylloide* and showed consistent and unique pyranocoumarin profiles. A small number of latex samples was collected from other species of *Calophyllum*.

Results of the latex survey corroborated the extract studies. For example, latex samples from *C. canum* (3




trees), *C. nodosum* (2 trees), and two samples from *C. sclerophyllum* (*C. rhizophorum*) all lacked pyranocoumarins by TLC and/or $^1$H NMR. In latex samples from *C. lanigerum* (43 trees), the only pyranocoumarin present in significant amounts was the tricyclic compound **7**. It was present in high quantities, clearly dominating the $^1$H NMR spectra of the crude latex. As with the organic extracts obtained from *C. teysmannii* var. *inophylloide*, the latex samples fell into two distinct groups. The first group contained almost exclusively (−)-calanolide B (**2**) and soulattrolide (**8**) in a 3:2 ratio. There were also other pyranocoumarins present in very small quantities (e.g., calanolide A has been detected as ca. 0.05% crude latex extract). All but two of these samples were collected from trees in the Gunong Pueh Forest Reserve, Semansan, Sarawak, Malaysia. A second group of latex samples from *C. teysmannii* var. *inophylloide* (6 trees) contained calanone (**14**), soulattrolide (**8**), and soulattolone (**13**) in the latex sample. These samples were collected from at least three different sites in Sarawak. These results suggest that there may be two distinct chemotypes for the species *C. teysmannii* var. *inophylloide*.

Relationships within *Calophyllum* are not well understood; although there are some groups of clearly related species, the overall morphological variation in the genus is in many ways slight.[26] Species from the New World are not markedly distinct from those in the Old World. The NCI Repository collection contains samples from much of the geographical and morphological range of the species, including representatives of one distinctive Papuasian group with angled stones (*C. papuanum*, *C. pauciflorum*). Because most Papuasian (and Pacific) species are not immediately related to those from West Malesia, it is premature to speculate as to whether species with coumarins will be found in the former areas. Although the six species containing coumarins are neither members of the same species group nor otherwise immediately related, it is interesting to see that where more obviously related species have been sampled, they often score negative (e.g., *C. papuanum*/*C. pauciflorum*; *C. ferrugineum*/*C. sundaicum*; *C. mariae*/*C. brasiliensis*). Some species groups are mixed. For example *C. nodosum* is positive, while *C.* aff. *nodosum*, and *C.* cf. *depressinervosum* are negative; furthermore, the presence of coumarins is not always consistent within a species. The presence of coumarins could characterize species groups within *Calophyllum*. The stable, consistent composition of the latex samples used in the study suggests that they may be very useful as infrageneric and infraspecific chemotaxonomic markers, as well as renewable sources of potential anti-HIV agents.

In addition, it is clear that anti-HIV data for the *Calophyllum* extracts cannot be used as a reliable marker for the presence of pyranocoumarins. This is due to the fact that only one of the three structural types observed, tetracylic pyranocoumarins with a *gem*-dimethyl group on the C ring and a $\beta$-OH or ketone at position 12, has anti-HIV activity, while the others are inactive. The presence of active extracts that did not contain pyranocoumarins is also not surprising given that the extracts and fractions were not treated to remove tannins.

## Experimental Section

**General Experimental Procedures.** NMR spectra were recorded on a Varian VXR 500 spectrometer using $CDCl_3$ as solvent and internal standard. TLC studies were done using Si gel plates in hexane−EtOAc (7:3). Plates were air-dried and then sprayed with vanillin−$H_2SO_4$ and heated for 30 s. Full color development occurred over the course of 8−12 h.

**Plant Material.** All plant materials were collected under contract for the NCI. Herbarium specimens are deposited at the Smithsonian Institution, Washington, DC, and at the herbarium of the contract organization—the Field Museum, Harvard University Herbaria for the University of Illinois at Chicago, Missouri Botanical Garden, or New York Botanical Garden.

**Extract Selection and Extraction.** All organic *Calophyllum* extracts available in the NCI Natural Product Repository before January 1996, were surveyed by TLC for the presence of pyranocoumarins and tested in the NCI's primary HIV screen for antiviral activity. Representative extracts of each *Calophyllum* species, which were positive for pyranocoumarins by TLC, were selected for further chemical exploration. The organic extract (200 mg) was dissolved in a small volume of 70% aqueous MeOH (25−50 mL) and partitioned with $CH_2Cl_2$ (3 × 50 mL). The $CH_2Cl_2$ fractions were combined and evaporated to dryness; a $^1$H NMR spectrum of each $CH_2Cl_2$ fraction was obtained and analyzed for the presence of pyranocoumarins, while the fraction was tested with the extract in the primary anti-HIV screen. Representative fractions that

contained signals indicative of pyranocoumarins ($\delta$ 6.8−6.6 d, H8; $\delta$ 5.8−6.0 s, H3; $\delta$ 5.6−5.4 d, H7) were selected for isolation of the pyranocoumarins present. Isolation of the pyranocoumarins from each *Calophyllum* species is detailed only once, unless collections contained different compounds. The isolation of the individual compounds has been described elsewhere.[12,29,30]

**Isolation of compounds from *Calophyllum lanigerum*. Q66O0351(N10813):** 5-g portion of the extract yielded calanolide E (**7**, 198 mg, 4.0%); its diastereomer, calanolide E2 (**9**, 30 mg, 0.6%); and cordatolide E (**10**, 60 mg, 1.2%). Details of the isolation and spectral data for the compounds have been described elsewhere.[12,29]

**Q67I1478 [Q67I1478 (N46637), Q67I1476 (N46639), Q67I1477 (N46641)]:** the organic crude extract (2.3 g) yielded pseudocordatolide C (**12**, 17 mg, 0.7%). Spectral data for **12** have been reported.[29]

**U44Z6993 [U44Z6993 (N45273), U44Z6694 (N45275)]:** the organic extract (2.5 g) yielded calanolide A (**1**, 11 mg, 0.44%) and cordatolide A (**4**, 13 mg, 0.5%). Physical data, including optical rotations, matched the data reported in the literature for these compounds.[12,24]

**Pyranocoumarins Isolated from *Calophyllum* aff. *pervillei*. Q66O1000 [Q66V1000 (N39205), Q66V1001 (N39187), Q66V1002 (N39171)]:** the organic extract (500 mg) yielded calophyllolide (**15**, 8.5 mg, 1.7%). Physical data, including optical rotations, matched the data reported in the literature for these compounds.[31,32]

**Pyranocoumarins Isolated from *Calophyllum molle*. Q66O4175 [Q66O4175 (N43515), Q66O5149 (N19607)]:** the organic extract (5.5 g) yielded calanolide E (**7**, 27 mg, 2.4%). All spectral data and the optical rotation of the compound were consistent with the published data.[12]

**Pyranocoumarins Isolated from *Calophyllum aff. biflorum*. Q66O2760 [Q66O2760 (N5523), Q66O2759 (N26825)]:** the organic extract (250 mg) yielded calanone (**14**, 2.2 mg, 0.88%). Spectral data and the optical rotation obtained were identical to those reported in the literature.[30]

**Pyranocoumarins Isolated from *Calophyllum teysmannii*. U44Z4468 (N38055):** the crude organic extract (2.5 g) yielded calanolide F (**11**, 13.4 mg, 0.5%). Spectral data and the optical rotation obtained were identical to those reported in the literature.[29]

**Bioassay Procedures.** The anti-HIV assay utilized the Hatian variant of HIV, HTLV-III$_{RF}$ in CEM-SS human lymphoblastoid cells, as described elsewhere.[28]

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## References and Notes

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