

**ANTI HIV-1 ACTIVE CALOPHYLLUM COUMARINS:  
DISTRIBUTION, CHEMISTRY, AND ACTIVITY**

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**Abstract-** Some dipyranocoumarins isolated from *Calophyllum* genus, Guttiferae (Clusiaceae), show anti HIV-1 activity. The HIV-1 active *Calophyllum* coumarins such as (+)-calanolide A [(+)-**1**] and (+)-inophyllum B [(+)-**13**] have a (2*R*,3*S*,4*S*)-2,3-dimethyl-4-chromanol ring as common structural requirements, whereas closely related coumarins were significantly less active or totally inactive. Thus, the stereochemistry of the chromanol ring in *Calophyllum* coumarins could be responsible for anti HIV-1 activity. Calanolide A [(+)-**1**] has currently been included in clinical trials. This review describes the distribution, chemistry, and the anti HIV-1 activity of *Calophyllum* coumarins.

## 1. Introduction

In 1992 the research group of National Cancer Institute (NCI) reported the isolation of a series of 4-propyldipyranocoumarins, the calanolides, from an organic extract of the tropical rainforest tree *Calophyllum lanigerum* var. *austrororiaceum*, Guttiferae (Clusiaceae), as active constituents during programs aimed at acquiring and screening extracts from natural sources for anti-HIV activity.<sup>1</sup> (+)-Calanolide A [(+)-**1**] among the isolated coumarins was demonstrated to be strongly active against HIV-1. Evaluation of its activity against HIV-1 reverse transcriptase (RT) and non-nucleoside reverse transcriptase inhibitor (NNRTI)-resistant viruses,<sup>2</sup> as well as detailed enzyme kinetics for RT inhibition,<sup>3</sup> suggested that (+)-**1** represented a novel class of HIV-1 specific RT inhibitor.<sup>2</sup> Currently (+)-calanolide A [(+)-**1**] has been in clinical trials to evaluate its safety and pharmacokinetics as single and multiple doses in both normal healthy and HIV-infected volunteers.<sup>4</sup>

On the other hand, in 1993 the research group of SmithKline Beecham Pharmaceuticals reported that the acetone extract of the giant African snail, *Achatina fulica*, showed activity against HIV-1 RT.<sup>5</sup> Fractionation of the extract yielded 4-phenyldipyranocoumarins of the inophyllum class, previously isolated from methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) extract of *C. inophyllum* which is known as the source of nutrition for *A. fulica*. Thus, examination of the CH<sub>2</sub>Cl<sub>2</sub>-MeOH extract of *C. inophyllum* resulted in the isolation of the same natural products in considerably greater yield. Specifically (+)-inophyllums B [(+)-**13**] and P [(+)-**14**] among the isolated coumarins inhibited HIV RT and both were active against HIV-1 in cell culture.<sup>5</sup> The HIV-1 active *Calophyllum* coumarins such as (+)-calanolide A [(+)-**1**] and (+)-inophyllum B [(+)-**13**]

have a (2*R*,3*S*,4*S*)-2,3-dimethyl-4-chromanol ring as a common structural requirement in their molecules, whereas closely related coumarins were significantly less active or totally inactive. Therefore, the stereochemistry of the chromanol ring in *Calophyllum* coumarins appears to be responsible for anti HIV-1 activity. In this article we review publications of the distribution, chemistry, and the biological activity of *Calophyllum* coumarins which have appeared previously May 1999. Synthetic works on these coumarins will not be included because of no additional work subsequent to the recent review.<sup>6</sup>

## 2. Structural Classification

*Calophyllum* coumarins can be classified into three groups with respect to the C4 substituent on the lactone ring of the coumarin, where n-propyl (calanolides; Figure 1), phenyl (inophyllums; Figure 2), and methyl groups (cordatolides; Figure 3) have been encountered. These compounds fall into four basic structural classes: (i) tetracyclic dipyrancoumarins in which the C rings have a *gem*-dimethyl group (Class I: Tetracyclic dipyrancoumarins); (ii) tetracyclic dipyrancoumarins with reversed C and D pyran rings (Class II: Reverse tetracyclic dipyrancoumarins, the *gem*-dimethyl groups being found in the D ring); (iii) tricyclic pyranocoumarins (Class III: Tricyclic pyranocoumarins which contain a noncyclized equivalent of either the C or the D rings of the tetracyclic structure class); (iv) coumarins without an additional ring (Class IV). Interestingly, (+)-calanolides E1 [(+)-**11**] and E2 [(+)-**12**] in the calanolides and (+)-cordatolide E [(+)-**41**] in the cordatolides, belonging to Class III, are dihydrocoumarins in which the C3/C4 double bond is fully saturated.

Coumarins in Classes I and II can be further divided into two subgroups, being either chromanol-coumarins with a chromanol ring or chromanone-coumarins with a chromanone ring, dependent upon the character of the oxygen function at C4 in 2,3-dimethylpyran rings.

(+)-Pseudocalanolides C [(+)-**7**] and D<sup>7</sup> [(+)-**8**] were previously reported as calanolides C and D<sup>1</sup> respectively, belonging to Class I in which the C ring has a *gem*-dimethyl group. However, there was an apparent discrepancy in the <sup>1</sup>H NMR spectrum of synthetic calanolide C<sup>8</sup> with that of the natural product. The natural compound had an observed chemical shift of 6.83 for 8-H, while the corresponding proton resonance of the synthetic material reportedly was observed at 6.63. <sup>1</sup>H NMR spectra of a synthetic structure corresponding to this initially reported for natural compound calanolide C showed some subtle differences from those of the natural product. Further analysis has resulted in revision of the stereostructures of the natural compounds, now renamed pseudocalanolides C and D, found to belong to Class II in which the D ring has a *gem*-dimethyl group.

The structures of apetatolide (**29**), a linear type tricyclic inophyllum coumarin, and oblongulide (**40**), an angular type tricyclic cordatolide coumarin, were similarly revised by comparison of data from synthetic coumarins<sup>8</sup> with those of proposed structures.

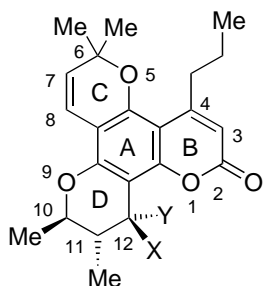
## 3. Distribution

The genus *Calophyllum* is a large group of tropical trees constituting of approximately 180-200 different species, mainly occurring in the Indo-Pacific region.<sup>9</sup> Natural coumarins isolated from *Calophyllum* species are summarized in Table 1.<sup>1,4,5,7,10-32</sup>

## I. Calanolide Series

### I-1. Class I: Tetracyclic dipyranocoumarins

#### Chromanol-coumarins



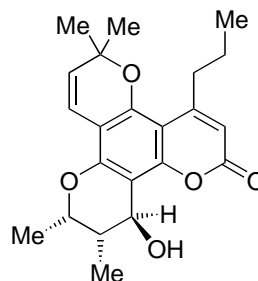
X=OH, Y=H : (+)-calanolide A [(+)-1]

X=OMe, Y=H : (+)-12-methoxycalanolide A [(+)-2]

X=OAc, Y=H : (+)-12-acetoxycalanolide A [(+)-3]

X=H, Y=OH : (+)-calanolide B [(+)-4] {The enantiomer : (-)-calanolide B (costatolide) [(-)-4]}

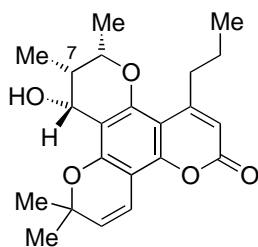
X=H, Y=OMe : (+)-12-methoxycalanolide B [(+)-5]



(-)-calanolide F [(-)-6]

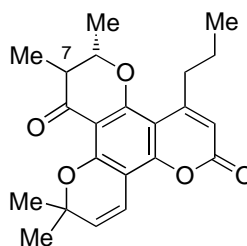
### I-2. Class II: Reverse tetracyclic dipyranocoumarins

#### Chromanol-coumarin



(+)-pseudocalanolide C [(+)-7]  
(after revision)

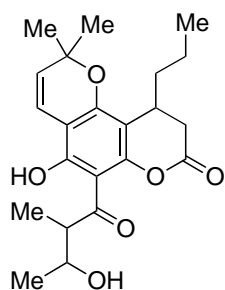
#### Chromanone-coumarins



7 $\alpha$ -Me : (+)-pseudocalanolide D [(+)-8] (after revision)

7 $\beta$ -Me : tomentolide B\* (9)

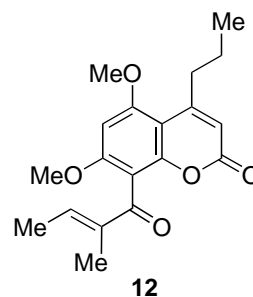
### I-3. Class III: Tricyclic pyrano(dihydro)coumarins



(+)-calanolides E1\* [(+)-10] and E2\* [(+)-11]

(calanolide E: a mixture of E1 and E2)

### I-4. Class IV: Others



12

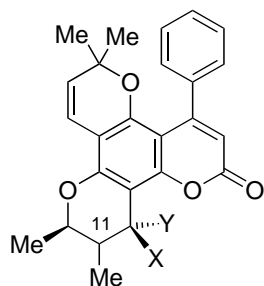
\* unknown absolute configuration

Figure 1. Classification of Natural *Calophyllum* coumarins: Calanolide series

## II. Inophyllum Series

### II-1. Class I: Tetracyclic dipyrano-coumarins

#### Chromanol-coumarins



11 $\alpha$ -Me

X=OH, Y=H : (+)-inophyllum B [(+)-**13**]

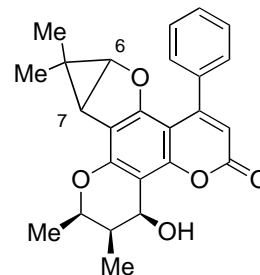
X=H, Y=OH : (+)-inophyllum P [(+)-**14**]

{The enantiomer : soulattrolide [(-)-*trans*-dihydroinophyllolide][(-)-**14**]}

11 $\beta$ -Me

X=H, Y=OH : (+)-inophyllum D [(+)-**15**]

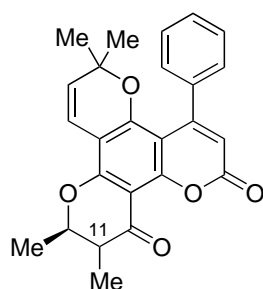
X=OH, Y=H : (+)-inophyllum A [(+)-*cis*-dihydroinophyllolide] [(+)-**16**]



6 $\alpha$ , 7 $\alpha$  : (+)-inophyllum G-1 [(+)-**17**]

6 $\beta$ , 7 $\beta$  : (-)-inophyllum G-2 [(-)-**18**]

#### Chromanone-coumarins



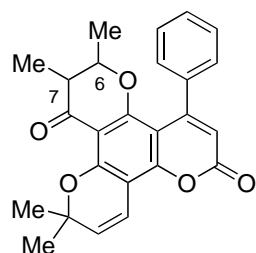
11 $\alpha$ -Me : (+)-inophyllum C [(+)-inophyllolide] [(+)-**19**]

{The enantiomer : (-)-*trans*-inophyllolide (soulattrolone)[(-)-**19**]}

11 $\beta$ -Me : (+)-inophyllum E [(+)-**20**]

### II-2. Class II: Reverse tetracyclic dipyrano-coumarin

#### Chromanone-coumarin



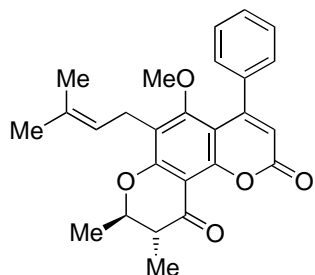
6,7-*trans* : tomentolide A\* (**21**)

\* unknown absolute configuration

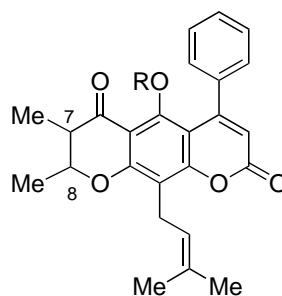
**Figure 2.** Classification of Natural *Calophyllum* coumarins: Inophyllum series (Part 1)

### II-3. Class III: Tricyclic pyranocoumarins

#### Chromanone-coumarins



(+)-teysmanone B [(+)-**22**]



7,8-*trans*

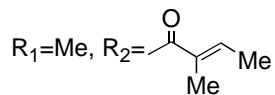
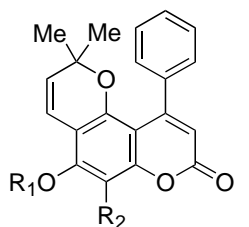
R=H: (-)-calaustralin\* [(-)-**23**]

R=Me: (-)-O-methylcalaustralin\* [(-)-**24**]

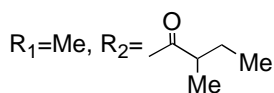
7,8-*cis*

R=Me: (-)-O-methylisocalaustralin\* [(-)-**25**]

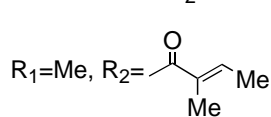
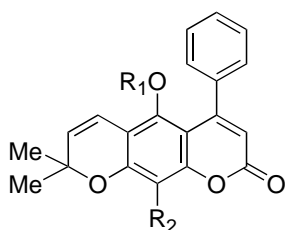
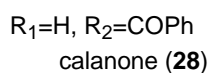
#### Others



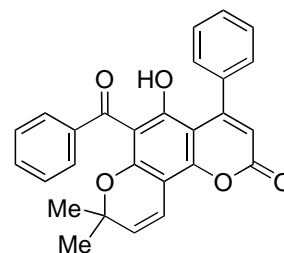
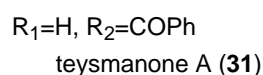
calophyllolide (**26**)



ponnalide E (**27**)

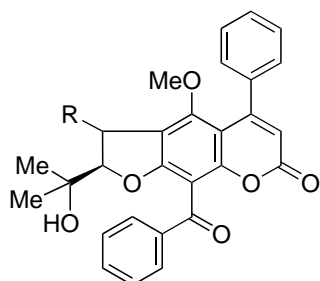


apetatolide (**29**) (after revision)



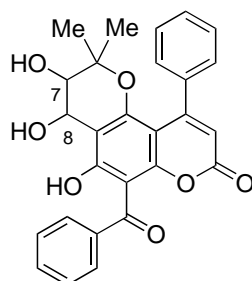
isocalanone (**30**)

### II-4. Others

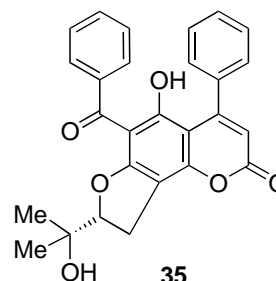


R=H (**32**)

R=OH\* (**33**)



7,8-*trans* **34\***



**35**

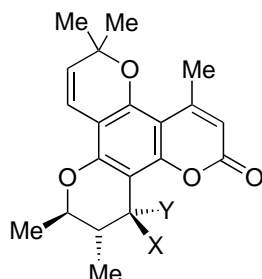
\* unknown absolute configuration

Figure 2. Classification of Natural *Calophyllum* coumarins: Inophyllum series (Part 2)

### III. Cordatolide Series

#### III-1. Class I: Tetracyclic dipyranocoumarins

##### Chromanol-coumarins



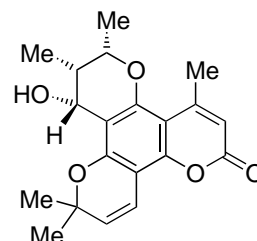
X=OH, Y=H : (+)-cordatolide A [(+)-**36**]

X=H, Y=OH : (-)-cordatolide B [(-)-**37**]

X=H, Y=OMe : 12-methoxycordatolide B\* (**38**)

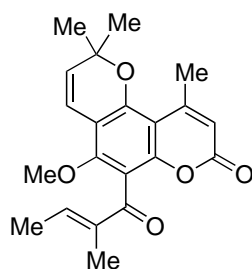
#### III-2. Class II: Reverse tetracyclic dipyranocoumarin

##### Chromanol-coumarin

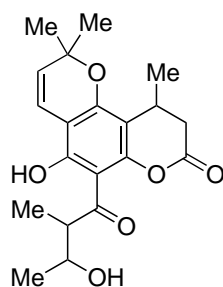


(+)-pseudocordatolide C\* [(+)-**39**]

#### III-3. Class III: Tricyclic pyrano(dihydro)coumarins



oblongulide (**40**)  
(after revision)



(+)-cordatolide E\* [(+)-**41**]

\* unknown absolute configuration

Figure 3. Classification of Natural *Calophyllum* coumarins: Cordatolide series

**The Calanolides.** Costatolide, which is now recognized to be (-)-calanolide B [(-)-**4**], was isolated in 41% yield as the first calanolide from a mixture of resin and bark exuded from an injured tree of *C. costatum* growing in northern Queensland, Australia in 1964.<sup>16</sup> Three years later it was reported that an optically inactive tomentolide B (**9**) appeared in *C. tomentosum* together with tomentolide A (**21**), an inophyllum coumarin.<sup>10</sup>

In 1992 anti-HIV bioassay-guided fractionation of an extract of *C. lanigerum* var. *austrocoriaceum* fruit and twigs collected in Sarawak, Malaysia, by the NCI group led to the isolation of eight new calanolides, (+)-calanolide A [(+)-**1**], (+)-12-methoxycalanolide A [(+)-**2**], (+)-12-acetoxycalanolide A [(+)-**3**], (+)-calanolide B [(+)-**4**], (+)-12-methoxycalanolide B [(+)-**5**], (+)-pseudocalanolide C<sup>7</sup> (so-called calanolide C) [(+)-**7**], (+)-pseudocalanolide D<sup>7</sup> (so-called calanolide D) [(+)-**8**], and (+)-calanolide E1 [(+)-**10**].<sup>1</sup> Among them (+)-calanolide A [(+)-**1**] and (+)-calanolide B [(+)-**4**] were completely protective against HIV-1 replication and cytopathicity (EC<sub>50</sub> values of 0.1 μM and 0.4 μM, respectively).

The best yields of (+)-calanolide A [(+)-**1**] obtained from the original collection were ~1 mg/g extract (of

**Table 1.** The source plants of *Calophyllum* coumarins (Part 1)

Species	Coumarins		
	Calanolides	Inophyllums	Cordatolides
<i>C. apetalum</i>		apetatolide <sup>10</sup> <b>(29)</b>	
<i>C. australium</i>		calaustralin <sup>11</sup> <b>(23)</b>	
<i>C. bracteatum</i>		calophyllolide <sup>12</sup> <b>(26)</b>	
<i>C. cerasiferum</i>	(-)-calanolide B (costatolide) <sup>13</sup> [(-)- <b>4</b> ]		
<i>C. cordato-oblongum</i>			(+)-cordatolide A <sup>14,15</sup> [(+)- <b>36</b> ] (-)-cordatolide B <sup>14,15</sup> [(-)- <b>37</b> ] 12-methoxycordatolide B <sup>15</sup> <b>(38)</b> oblongulide <sup>14,15</sup> <b>(40)</b>
<i>C. costatum</i>	costatolide [(-)-calanolide B] <sup>16</sup> [(-)- <b>4</b> ]		
<i>C. inophyllum</i>	no name <sup>17</sup> <b>(12)</b>	(+)-inophyllum B <sup>5,13,18</sup> [(+)- <b>13</b> ] (+)-inophyllum P <sup>5,13</sup> [(+)- <b>14</b> ] (+)-inophyllum D <sup>5,18</sup> [(+)- <b>15</b> ] (+)-inophyllum A [(+)- <i>cis</i> -dihydroinophyllolide] <sup>5,18</sup> [(+)- <b>16</b> ] (+)-inophyllum G-1 <sup>5</sup> [(+)- <b>17</b> ] (-)-inophyllum G-2 <sup>5</sup> [(-)- <b>18</b> ] (+)-inophyllum C [(+)-inophyllolide] <sup>5,13,18</sup> [(+)- <b>19</b> ] inophyllolide <sup>19</sup> <b>(19)</b> (+)-inophyllum E <sup>5,13,18</sup> [(+)- <b>20</b> ] (-)-calaustralin <sup>20</sup> [(-)- <b>23</b> ] calophyllolide <sup>13,21,22</sup> <b>(26)</b> ponnalide E <sup>17,23</sup> <b>(27)</b> apetalolide <sup>22</sup> <b>(29)</b>	

**Table 1.** The source plants of *Calophyllum* coumarins (Part 2)

Species	Coumarins		
	Calanolides	Inophyllums	Cordatolides
<i>C. lanigerum</i> var. <i>austrocoriaceum</i>	(+)-calanolide A <sup>1</sup> [(+)- <b>1</b> ] (+)-12-methoxycalanolide A <sup>1</sup> [(+)- <b>2</b> ] (+)-12-acetoxycalanolide A <sup>1</sup> [(+)- <b>3</b> ] (+)-calanolide B <sup>1</sup> [(+)- <b>4</b> ] (+)-12-methoxycalanolide B <sup>1</sup> [(+)- <b>5</b> ] (-)-calanolide F <sup>24</sup> [(-)- <b>6</b> ] (+)-pseudocalanolide C <sup>1,7,24</sup> [(+)- <b>7</b> ] (+)-pseudocalanolide D <sup>1,7,24</sup> [(+)- <b>8</b> ] (+)-calanolide E1 <sup>1,23,25</sup> [(+)- <b>10</b> ] (+)-calanolide E2 <sup>1,24</sup> [(+)- <b>11</b> ]		(+)-pseudocordatolide C <sup>24</sup> [(+)- <b>39</b> ] (+)-cordatolide E <sup>24</sup> [(+)- <b>41</b> ]
<i>C. molle</i>	calanolide E <sup>26</sup>		
<i>C. mooni</i>		(-)- <i>trans</i> -dihydroinophyllolide [(-)-inophyllum P; soulattrolide] <sup>27</sup> [(-)- <b>14</b> ] (+)- <i>cis</i> -dihydroinophyllolide [(+)-inophyllum A] <sup>27</sup> [(+)- <b>16</b> ]	
<i>C. aff. pervillei</i>		calophyllolide <sup>26</sup> ( <b>26</b> )	
<i>C. soulattri</i>		soulattrolide [(-)-inophyllum P; (-)- <i>trans</i> -dihydroinophyllolide] <sup>28</sup> [(-)- <b>14</b> ]	



**Table 1.** The source plants of *Calophyllum* coumarins (Part 3)

Species	Coumarins		
	Calanolides	Inophyllums	Cordatolides
<i>C. teysmannii</i> var. <i>inophyllolide</i>	(-)-calanolide B (costatolide) <sup>25</sup> [(-)- <b>4</b> ] (-)-calanolide F <sup>24</sup> [(-)- <b>6</b> ] no name <sup>24</sup> ( <b>12</b> ) calanolide A <sup>32</sup> ( <b>1</b> ) [as a scalemic mixture with an excess of (+)- <b>1</b> ]	soulattrolide [(-)-inophyllum P; (-)- <i>trans</i> -dihydroinophyllolide] <sup>25,29</sup> [(-)- <b>14</b> ] inophyllum C (inophyllolide) <sup>30,31</sup> ( <b>19</b> ) (-)- <i>trans</i> -inophyllolide (soulattrolone) <sup>29</sup> [(-)- <b>19</b> ] inophyllum E <sup>31</sup> ( <b>20</b> ) (+)-teysmanone B <sup>31</sup> [(+)- <b>22</b> ] (-)-O-methylcalaustralin <sup>30</sup> [(-)- <b>24</b> ] (-)-O-methylisocalaustralin <sup>30</sup> [(-)- <b>25</b> ] calanone <sup>29,30,31</sup> ( <b>28</b> ) isocalanone <sup>30</sup> ( <b>30</b> ) teysmanone A <sup>31</sup> ( <b>31</b> ) no name <sup>30</sup> ( <b>32</b> ) no name <sup>30</sup> ( <b>33</b> ) no name <sup>30</sup> ( <b>34</b> ) no name <sup>30</sup> ( <b>35</b> )	
<i>C. tomentosum</i>	tomentolide B <sup>10</sup> ( <b>9</b> )	tomentolide A <sup>10</sup> ( <b>21</b> )	

leaves), meaning that quite large collections of leaves would be required to obtain sufficient supplies. Thus, the NCI group focused on latexes because of easy collection of them by making several small slash wounds in the bark of mature trees and the reavailability of plant sources after the wounds healed. However, the latex from numerous specimens of *C. lanigerum* var. *austrocoriaceum* contained no discernible amounts of calanolide A [(+)-**1**], nor was the crude latex active in the anti-HIV assay,<sup>25</sup> the major component being (+)-calanolide E1 [(+)-**10**]. On the other hand, the latex of *C. teysmanni* var. *inophylloide* was active against HIV, appearing (TLC, NMR) to contain significant amounts of (-)-calanolide B [(-)-**4**] (48% of the latex extractable), and being intermediate in potency between (+)-calanolide A [(+)-**1**] and (+)-calanolide B [(+)-**4**]. Based on its substantial anti-HIV activity and potential access to larger quantities by sustained harvesting of latex, (-)-calanolide B [(-)-**4**] is being explored as a possible alternative development candidate to (+)-calanolide A [(+)-**1**].

During resolution works of the enantiomers of calanolides A (**1**) and B (**4**) what appeared to be (+)-calanolide A [(+)-**1**] was isolated as a minor component (<0.1%) of the latex of *C. teysmanni* var. *inophylloide*, but the observed specific rotation of this material was low,  $[\alpha]_D +15.1^\circ$  vs.  $[\alpha]_D +60^\circ$  reported for (+)-calanolide A [(+)-**1**]. Chiral analysis revealed that the calanolide A fraction from this latex was a scalemic mixture of calanolides A (**1**), with an enantiomeric excess of (+)-**1**.<sup>32</sup> Natural calanolide B<sup>1</sup> (**4**) isolated as pure (+)-form,  $[\alpha]_D +10^\circ$ , was also found to be a scalemic mixture containing a slight excess of the (+)-enantiomer.

A chemotaxonomic survey of *Calophyllum* extracts showed that four new pyranocoumarins had been isolated from extracts of *C. lanigerum* var. *austrocoriaceum* and *C. teysmanni* var. *inophylloide*.<sup>24</sup> (+)-Calanolides E1 [(+)-**10**] and E2 [(+)-**11**] were isolated together with (+)-cordatolide E [(+)-**41**], a cordatolide, from the stem bark and (+)-pseudocalanolide C [(+)-**7**] from the leaves of the former plant, respectively. In addition, the leaves and twigs of the latter plant contained (-)-calanolide F [(-)-**6**] and the latex contained a coumarin (**12**), which had been isolated from *C. inophyllum* in 1972, in addition to (-)-calanolide B [(-)-**4**].

It has been reported that *C. cerasiferum* contains (-)-calanolide B [(-)-**4**] as its major coumarin constituent in significant amounts (0.4 g/kg of the crushed seeds) and thus could constitute a renewable source of this compound.<sup>13</sup> Since the original source of (+)-calanolide A [(+)-**1**] was destroyed, finding new sources of active pyranocoumarins has become a worthwhile endeavour.

Recently the NCI group reported 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.<sup>26</sup> 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 contained prenylated coumarins, suggesting that these compounds, while sometimes abundantly present, are not widespread in the genus. These eight species are *C. inophyllum*, *C. lanigerum* var. *austrocoriaceum*, *C. molle*, *C. nodasum*, *C. aff. pervillei*, *C. soulattri*, *C. tecamahaca*, and *C. teysmanni*. A detailed analysis of coumarins present in several of the coumarin-positive extracts from *C. lanigerum*, *C. molle*, *C. aff. pervillei*, and *C. teysmanni*. Representative samples of the TLC-positive extracts were partitioned between CH<sub>2</sub>Cl<sub>2</sub> and 25% aqueous

MeOH; the CH<sub>2</sub>Cl<sub>2</sub>-soluble materials were then analyzed by TLC and <sup>1</sup>H NMR to confirm the presence of pyranocoumarins.

Three calanolides of the calanolide E group [a mixture of calanolides E1 (**10**) and E2 (**11**), calanolide E2 (**11**), and calanolide F (**6**)] were isolated from 16 extracts of *C. lanigerum* together with three cordatolides [cordatolide A (**36**), pseudocordatolide C (**39**), and cordatolide E (**41**)]. Calanolide E and pseudocordatolide C (**39**) were commonly found in those extracts. No coumarins other than calanolide E were found in *C. molle*. Extracts of *C. aff. pervillei* and *C. teysmanni* var. *inophylloide* also showed little variation in their pyranocoumarin metabolites. The former contains no calanolides, but an inophyllum, calophyllolide (**26**). From the latter (-)-calanolide B [(-)-**4**] was identified as a calanolide coumarin and soulattrolide [(-)-*trans*-dihydroinophyllolide] [(-)-**14**], soulattrone [(-)-*trans*-inophyllolide] [(-)-**19**], and calanone (**28**) were identified as inophyllum coumarins. The presence of two chemotypes of *C. teysmanni* var. *inophylloide* was suggested; one produces large amounts of (-)-calanolide B [(-)-**4**] and soulattrolide [(-)-**14**],<sup>25</sup> the other containing calanone (**28**) as the preliminary constituent along with minor amounts of soulattrolone [(-)-**19**].<sup>29</sup>

**The Inophyllums:** Inophyllums have a longer history than calanolides. In 1951 calophyllolide (**26**) was isolated from *C. inophyllum* as the progenitor of this structural class.<sup>7,21</sup> Six years later its structure was determined to be that of a tricyclic pyranocoumarin (Class III) and, at the same time, that of inophyllolide (**19**) was also determined to be that of a tetracyclic dipyrancoumarin (Class I) with a chromanone ring (chromanone-coumarin).<sup>21,24</sup>

In 1968 five typical inophyllums, inophyllums B (**13**), D (**15**), A (**16**), C (**19**), and E (**20**) were isolated from *C. inophyllum* as chemical components with piscicidal activity.<sup>18</sup> Soulattrolide [(-)-*trans*-dihydroinophyllolide] [(-)-**14**], corresponding to an enantiomer of (+)-inophyllum P [(+)-**14**], has been obtained from three different species of *C. mooni*,<sup>27</sup> *C. soulattri*,<sup>28</sup> and *C. teysmannii*.<sup>25,29</sup> The species of *C. teysmannii* contains calanolides, costatolide [(-)-calanolide B]<sup>25</sup> [(-)-**4**] as well as calanolide F<sup>24</sup> (**6**), as mentioned above.

In 1993 bioassay-guided fractionation of the acetone extract of the giant African snail, *Achatina fulica*, collected in the Seychelles, was found to show anti HIV RT activity.<sup>5</sup> (+)-Inophyllums B [(+)-**13**], A [(+)-**16**], C [(+)-**19**], and E [(+)-**20**] and calophyllolide (**26**) were isolated as coumarin derivatives with potent inhibitory activity. They belong to a known class of coumarins, previously isolated from the leaves of *C. inophyllum*. Since the snail is known to feed on leaves on the *Calophyllum* plant, the dried leaves and twigs of *C. inophyllum* were examined for the separation of chemical components. Bioassay-guided fractionation of the extract led to the isolation and characterization of the five compounds isolated from *A. fulica* plus three novel compounds: (+)-inophyllum P [(+)-**14**] {corresponding to the 12-epimer of (+)-inophyllum B [(+)-**13**]}, (+)-inophyllum G-1 [(+)-**17**], and (-)-inophyllum G-2 [(-)-**18**].

During 1970<sup>11,12,17,22,23</sup> tricyclic pyranocoumarins with either a chromanone ring such as (-)-calaustralin [(-)-**23**] or a 2,2-dimethylchromene skeleton such as apetatolide (**29**) [an isomer of calophyllolide (**26**)] were isolated. Recent examination<sup>30,31</sup> of *C. teysmannii* showed the presence of several new tricyclic pyranocoumarins such as compounds (**32-35**).

**The Cordatolides:** These represent a small class of *Calophyllum* coumarins and only six members are

known: (+)-cordatolide A [(+)-**36**], (-)-cordatolide B [(-)-**37**], 12-methoxycordatolide B (**38**), and oblongulide (**40**) from *C. cordato-oblongum*<sup>14,15</sup> and (+)-pseudocordatolide C [(+)-**39**] and (+)-cordatolide E [(+)-**41**] from *C. lanigerum*,<sup>24</sup> respectively. It is noteworthy that no isolation of a cordatolide with a chromanone ring (chromanone-coumarin) has been reported up to present.

#### 4. Chemistry: Stereochemistry and Conformational Analysis

The structures of *Calophyllum* coumarins were generally determined by conventional spectroscopic means. The relative stereochemistry and conformational analysis of the 2,3-dimethyl-4-chromanol rings in the chromanol-coumarins and the 2,3-dimethyl-4-chromanone rings in the chromanone-coumarins were deduced based upon the coupling constants between the ring protons in the <sup>1</sup>H NMR spectra and NOE enhancements. The stereochemistry can be also assignable by chemical correlation, for which either CrO<sub>3</sub> oxidation of chromanol derivatives or NaBH<sub>4</sub> reduction of chromanone derivatives is available.

Numbering of the chromanol or chromanone ring in the *Calophyllum* coumarins is different among Classes I-III. Therefore, numbering for a simple chromanol or chromanone ring system will be applied in the following discussion.

##### The Chromanol-Coumarins

Possible four diastereomeric forms of a 2,3-dimethyl-4-chromanol ring are found in the chromanol-coumarins (Table 2).

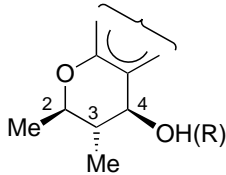
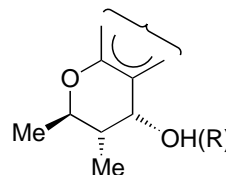
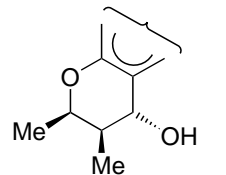
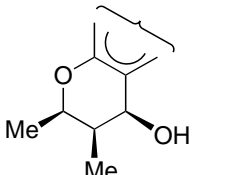
##### (I) 2,3-*trans*-3,4-*trans* Configuration

The anti HIV-1 active (+)-calanolide A [(+)-**1**], its methyl ether [(+)-**2**], and its acetate [(+)-**3**] in calanolides, (+)-inophyllum B [(+)-**13**] in inophyllums, and (+)-cordatolide A [(+)-**36**] in cordatolides contain a *trans, trans*-2,3-dimethyl-4-chromanol ring in their molecules. The stable conformations of such chromanol rings with *trans, trans* configuration are dependent upon the nature of the substituent at C4.

In the <sup>1</sup>H NMR spectrum of (+)-calanolide A [(+)-**1**], the 4-H benzylic carbinol proton in a chromanol ring showed an 8.0 Hz coupling to 3-H, which revealed that these two protons had a *trans* diaxial orientation.<sup>1</sup> A 9.0 Hz coupling between 2-H and 3-H established that 2-H also was axial. This assignment was supported by NOE enhancements (3%) observed between the diaxial 2-H and 4-H protons. Thus, (+)-calanolide A [(+)-**1**] was found to be a diastereoisomer of costatolide [(-)-calanolide B] [(-)-**4**], which showed  $J_{2,3}$  and  $J_{3,4}$  of 10.5 and 3.5 Hz, respectively.<sup>16</sup> Two related coumarin derivatives, (+)-inophyllum B [(+)-**13**] and (-)-cordatolide [(-)-**36**] reportedly had the same relative stereochemical features about the chromanol ring as those found in (+)-**1**, but differed in their C4 substituents. The  $J_{2,3}$  and  $J_{3,4}$  coupling constants observed in (+)-calanolide A [(+)-**1**] were in very close agreement with those reported for both compounds {e.g.  $J_{2,3}$ =9.1 Hz and  $J_{3,4}$ =7.8 Hz in (+)-inophyllum B<sup>5</sup> [(+)-**13**]}.<sup>1</sup>

In (+)-12-methoxycalanolide A [(+)-**2**] vicinal couplings were observed with coupling constants of  $J_{2,3}$ =3.5 Hz and  $J_{3,4}$ =3.7 Hz, respectively.<sup>1</sup> In addition a four-bond W coupling of 1.3 Hz was also observed between 2-H and 4-H, implying a pseudoequatorial configuration for 2-H and 4-H. Significant NOE enhancements between 3-H and both the C2 methyl group (3.5%) and the C4 methoxy group (3.5%) indicated that 3-H was *cis* to these two substituents and therefore had an equatorial orientation about the chromanol ring. It appears that in (+)-12-methoxycalanolide A [(+)-**2**], the preferred conformation of the

**Table 2.** Stereochemistries of the Chromanol Rings in the Chromanol-coumarins

	Types of Chromanols			
Coumarins	 <p>2,3-<i>trans</i>-3,4-<i>trans</i></p>	 <p>2,3-<i>trans</i>-3,4-<i>cis</i></p>	 <p>2,3-<i>cis</i>-3,4-<i>trans</i></p>	 <p>2,3-<i>cis</i>-3,4-<i>cis</i></p>
Calanolides	<p>(2<i>R</i>, 3<i>S</i>, 4<i>S</i>)                      (+)-calanolide A [(+)-<b>1</b>]                      (+)-12-methoxycalanolide A [(+)-<b>2</b>]                      (+)-12-acetoxycalanolide A [(+)-<b>3</b>]</p> <p>(2<i>S</i>, 3<i>R</i>, 4<i>R</i>)                      (-)-calanolide A [(-)-<b>1</b>]</p>	<p>(2<i>R</i>, 3<i>S</i>, 4<i>R</i>)                      (+)-calanolide B [(+)-<b>4</b>]                      (+)-12-methoxycalanolide B [(+)-<b>5</b>]</p> <p>(2<i>S</i>, 3<i>R</i>, 4<i>S</i>)                      (-)-calanolide B (costatolide) [(-)-<b>4</b>]</p>	<p>(2<i>R</i>, 3<i>R</i>, 4<i>R</i>)                      no coumarin</p> <p>(2<i>S</i>, 3<i>S</i>, 4<i>S</i>)                      (-)-calanolide F [(-)-<b>6</b>]</p>	<p>(2<i>R</i>, 3<i>R</i>, 4<i>S</i>)                      no coumarin</p> <p>(2<i>S</i>, 3<i>S</i>, 4<i>R</i>)                      (+)-pseudocalanolide C [(+)-<b>7</b>]</p>
Inophyllums	<p>(2<i>R</i>, 3<i>S</i>, 4<i>S</i>)                      (+)-inophyllum B [(+)-<b>13</b>]</p> <p>(2<i>S</i>, 3<i>R</i>, 4<i>R</i>)                      no coumarin</p>	<p>(2<i>R</i>, 3<i>S</i>, 4<i>R</i>)                      (+)-inophyllum P [(+)-<b>14</b>]</p> <p>(2<i>S</i>, 3<i>R</i>, 4<i>S</i>)                      soulattrolide                      ((-)-<i>trans</i>-dihydroinophyllolide)                      [(-)-<b>14</b>]</p>	<p>(2<i>R</i>, 3<i>R</i>, 4<i>R</i>)                      (+)-inophyllum D [(+)-<b>15</b>]</p> <p>(2<i>S</i>, 3<i>S</i>, 4<i>S</i>)                      no coumarin</p>	<p>(2<i>R</i>, 3<i>R</i>, 4<i>S</i>)                      (+)-inophyllum A                      [(+)-<i>cis</i>-dihydroinophyllolide] [(+)-<b>16</b>]                      (+)-inophyllum G-1 [(+)-<b>17</b>]                      (-)-inophyllum G-2 [(-)-<b>18</b>]</p> <p>(2<i>S</i>, 3<i>S</i>, 4<i>R</i>)                      no coumarin</p>
Cordatolides	<p>(2<i>R</i>, 3<i>S</i>, 4<i>S</i>)                      (+)-cordatolide A [(+)-<b>36</b>]</p> <p>(2<i>S</i>, 3<i>R</i>, 4<i>R</i>)                      no coumarin</p>	<p>(2<i>R</i>, 3<i>S</i>, 4<i>R</i>)                      no coumarin</p> <p>(2<i>S</i>, 3<i>R</i>, 4<i>S</i>)                      (-)-cordatolide B [(-)-<b>37</b>]</p> <p><i>unknown</i>                      12-methoxycordatolide B (<b>38</b>)</p>	<p>(2<i>R</i>, 3<i>R</i>, 4<i>R</i>)                      no coumarin</p> <p>(2<i>S</i>, 3<i>S</i>, 4<i>S</i>)                      no coumarin</p>	<p>(2<i>R</i>, 3<i>R</i>, 4<i>S</i>)                      no coumarin</p> <p>(2<i>S</i>, 3<i>S</i>, 4<i>R</i>)                      no coumarin</p> <p><i>unknown</i>                      (+)-pseudocordatolide C [(+)-<b>39</b>]</p>

chromanol ring is inverted relative to (+)-calanolide A [(+)-**1**]. Thus, in (+)-**1** all three protons of 2-H, 3-H, and 4-H were axial, and in (+)-**2** they were all equatorial.

In (+)-12-acetoxycalanolide A<sup>1</sup> [(+)-**3**] the coupling constants of  $J_{2,3}=6.5$  Hz and  $J_{3,4}=6.0$  Hz supported a pseudoaxial orientation of the chromanol ring protons, with its slightly diminished chromanol proton couplings indicating a slight twisting of the flexible chromanol ring. Further evidence for the proposed configuration was provided by 2% NOE enhancements between 2-H and 4-H.

### (II) 2,3-*trans*-3,4-*cis* Configuration

A *trans*, *cis*-2,3-dimethyl-4-chromanol ring system appears in both enantiomers of calanolide B (**4**) and (+)-12-methoxycalanolide B [(+)-**5**] in calanolides, both enantiomers of inophyllum P [*trans*-dihydroinophyllolide (**14**)] in inophyllums, and (-)-cordatolide B [(-)-**37**] in cordatolides.

In the <sup>1</sup>H NMR spectra of (+)-calanolide B<sup>1</sup> [(+)-**4**] proton-proton coupling constant analysis showed  $J_{2,3}=10.5$  Hz and  $J_{3,4}=3.3$  Hz. Thus, 2-H and 3-H are *trans* diaxial while 3-H and 4-H are in a *cis* configuration with 4-H in a pseudoequatorial orientation.

The 2-H, 3-H, and 4-H in (+)-inophyllum P [(+)-**14**] resonate at 4.29, 1.79, and 5.04, respectively.<sup>5</sup> Compared to (+)-inophyllum B [(+)-**13**] with a 2,3-*trans*-3,4-*trans* configuration the 2-H and 4-H appear *ca.* 0.3 ppm downfield while 3-H is *ca.* 0.2 ppm upfield. (+)-Inophyllum P [(+)-**14**] shows a large coupling constant ( $J=10.6$  Hz) and relatively small NOE enhancements (2-6%) between 2-H and 3-H, and a small coupling constant ( $J=3.3$  Hz) and large NOE enhancements (17-21%) between 3-H and 4-H, respectively, in the <sup>1</sup>H NMR spectra.

### (III) 2,3-*cis*-3,4-*trans* Configuration

A *cis*, *trans*-2,3-dimethyl-4-chromanol ring system appears in only two coumarins, (-)-calanolide F [(-)-**6**] in the calanolide series and (+)-inophyllum D [(+)-**15**] in the inophyllums.

The relative stereochemistry of (-)-calanolide F [(-)-**6**] was determined by coupling constant analysis and comparison with known compounds.<sup>24</sup> The small coupling observed between 2-H and 3-H ( $J=2.0$  Hz) and between 3-H and 4-H ( $J=2.0$  Hz) suggested that there were no *trans* diaxial relationships between neighboring protons. Thus, the molecule must have an axial-equatorial-axial or equatorial-axial-equatorial relationship among 2-H, 3-H, and 4-H. This is the same relative stereochemistry reported for (+)-inophyllum D [(+)-**15**]. Indeed, the coupling constants of (-)-calanolide F [(-)-**6**] closely match those reported for (+)-inophyllum D<sup>5,18</sup> [(+)-**15**] ( $J_{2,3}=2.0$  Hz and  $J_{3,4}=2.2$  Hz).

### (IV) 2,3-*cis*-3,4-*cis* Configuration

(+)-Pseudocalanolide C [(+)-**7**] in the calanolides, (+)-inophyllum A [(+)-*cis*-dihydroinophyllolide] [(+)-**16**], (+)-inophyllum G-1 [(+)-**17**], and (-)-inophyllum G-2 [(-)-**18**] in the inophyllums, and (+)-pseudocordatolide C [(+)-**39**] in the cordatolides all possess a *cis*, *cis*-2,3-dimethyl-4-chromanol ring.

The 2,3-*cis*-3,4-*cis* configuration of the chromanol ring in (+)-pseudocalanolide C<sup>7</sup> [(+)-**7**], which has been previously reported as calanolide C, was deduced as follows<sup>1</sup>: (i) the only notable difference between the <sup>1</sup>H and <sup>13</sup>C NMR spectra of (+)-pseudocalanolide C [(+)-**7**] and those recorded for calanolide A [(+)-**1**] were the resonance associated with the chromanol ring. The  $J_{2,3}$  in (+)-**7** was 2.5 Hz while  $J_{3,4}$  was 6.0 Hz. These coupling constants were insufficient to define the relative stereochemistry of C2, C3, and C4. However, the C4 hydroxyl proton exhibited a 1.5 Hz coupling to 4-H, which suggested that the rate of

exchange of the OH proton was reduced due to hydrogen bonding to O1. Hydrogen bonding to O1 would require an equatorial OH at C4. (ii) 5% NOE enhancements between 2-H and 4-H confirmed that these protons each had axial orientations. Therefore, 3-H had to be equatorial because of the small  $J_{2,3}$  coupling. (+)-Pseudocalanolide C [(+)-**7**] was thus the C3 epimer of (+)-calanolide A [(+)-**1**] and had the same substitution pattern and relative stereochemistry about the chromanol ring as the coumarin derivative inophyllum A (**16**). The  $J_{2,3}$  (3.3 Hz) and  $J_{3,4}$  values (5.4 Hz) reported for inophyllum A<sup>18,27</sup> (**16**) were in good agreement with the respective couplings observed in (+)-pseudocalanolide C [(+)-**7**].

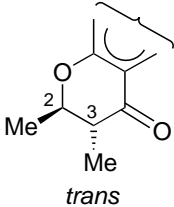
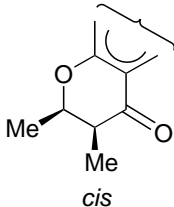
### The Chromanone-Coumarins

Two possible diastereomeric forms of a 2,3-dimethyl-4-chromanone ring are found in chromanone-coumarins in both calanolides and inophyllums, whereas there have been no reports in the cordatolide series (Table 3).

#### (I) *trans* Configuration

A *trans*-2,3-dimethyl-4-chromanone ring system appears in tomentolide B (**9**), in calanolides. In the inophyllum series both enantiomers of inophyllum C (inophyllolide) (**19**), tomentolide A (**21**), (+)-

**Table 3.** Stereochemistries of the Chromanone Rings in the Chromanone-coumarins

Coumarins	Types of Chromanones	
		
Calanolides	(2 <i>R</i> , 3 <i>R</i> ) no coumarin	(2 <i>R</i> , 3 <i>S</i> ) no coumarin
	(2 <i>S</i> , 3 <i>S</i> ) tomentolide B ( <b>9</b> )	(2 <i>S</i> , 3 <i>R</i> ) (+)-pseudocalanolide D [(+)- <b>8</b> ]
Inophyllums	(2 <i>R</i> , 3 <i>R</i> ) (+)-inophyllum C [(+)-inophyllolide] [(+)- <b>19</b> ] (+)-teyamanone B [(+)- <b>22</b> ]	(2 <i>R</i> , 3 <i>S</i> ) (+)-inophyllum E [(+)- <b>20</b> ]
	(2 <i>S</i> , 3 <i>S</i> ) (-)- <i>trans</i> -inophyllolide (soulattrolone) [(-)- <b>19</b> ]	(2 <i>S</i> , 3 <i>R</i> ) no coumarin
	<i>unknown</i> tomentolide A ( <b>21</b> ) (-)-calaustralin [(-)- <b>23</b> ] (-)-O-methylcalaustralin [(-)- <b>24</b> ]	<i>unknown</i> (-)-O-methylsocalaustralin [(-)- <b>25</b> ]
Cordatolides	(2 <i>R</i> , 3 <i>R</i> ) no coumarin	(2 <i>R</i> , 3 <i>S</i> ) no coumarin
	(2 <i>S</i> , 3 <i>S</i> ) no coumarin	(2 <i>S</i> , 3 <i>R</i> ) no coumarin

teysmanone B [(+)-**22**], (-)-calaustralin [(-)-**23**], and (-)-O-methylcalaustralin [(-)-**24**] possess the same ring system. As an example, inophyllum C (inophyllolide) (**19**) exhibits a large coupling constant ( $J=11.1$  Hz) and a relatively small NOE (7%) between vicinal 2-H and 3-H protons, indicating a *trans* diaxial relationship between them.<sup>5</sup>

## (II) *cis* Configuration

A *cis*-2,3-dimethyl-4-chromanone ring system appears in (+)-pseudocalanolide D [(+)-**8**] in the calanolides and (+)-inophyllum E [(+)-**20**] and (-)-O-methylisocalaustralin [(-)-**25**] in the inophyllums.

The small coupling ( $J=3.0$  Hz) between 2-H and 3-H in (+)-pseudocalanolide D<sup>7</sup> [(+)-**8**], which was previously reported as calanolide D, indicates that at least one of these protons is equatorial. As a large coupling [*e.g.*  $J_{2,3}=11.1$  Hz in inophyllum C (**19**)] is reported in *trans*, *trans* derivatives, the relative stereochemistry of the 2-H and 3-H protons in (+)-**8** has to be *cis*. (+)-Inophyllum E [(+)-**20**] possesses small vicinal coupling constant ( $J_{2,3}=3.4$  Hz) and large NOE enhancements (19-20%) between 2-H and 3-H, indicating an axial-equatorial relationship between them.<sup>5</sup>

## 5. Absolute Stereochemistry

The absolute stereochemistry of chromanol-coumarins has been determined either by a modified Mosher's method, which utilizes anisotropic shifts induced in the <sup>1</sup>H NMR spectra of  $\alpha$ -methoxy- $\alpha$ -(trifluoromethyl)phenylacetic (MTPA) esters of secondary alcohols to define the absolute stereochemistry, or by X Ray analysis (see Table 2). Chemical correlation of chromanone-coumarins to chromanol-coumarins with known stereochemistry has also been applied to the establishment of their absolute stereochemistry (see Table 3).

The absolute configuration of C4 in the chromanol ring of calanolides was determined to be 4*S* in (+)-calanolide A [(+)-**1**] and 4*R* in (+)-calanolide B [(+)-**4**] by a modified Mosher's method,<sup>1</sup> although esterification of (+)-**1** proceeded very slowly (24 h reflux), possibly due to the conformation of the chromanol ring. The changes of coupling constants of  $J_{2,3}=9.0$  Hz and  $J_{3,4}=8.0$  Hz in (+)-**1** to smaller ones ( $J_{2,3}=2.5$  Hz and  $J_{3,4}=2.5$  Hz) showed that in MTPA ester the methyl and ester groups were flipped to an axial position from an equatorial position. In addition a four-bond W coupling of 1.5 Hz between 2-H and 4-H could also be observed. The anisotropic shifts induced in the MTPA esters indicated that the bulky MTPA group was sterically repelled by the coumarin ring lactone. Thus, the plane which divides the molecule's proton resonances into  $\Delta\delta$ -positive and  $\Delta\delta$ -negative did not cleanly bisect the dihydropyran ring through C4 and O1. In (+)-calanolide A [(+)-**1**] the dividing plane seems to come closer to the carbon possessing the *gem*-dimethyl group (C6 in the full structure) in the C ring and in (+)-calanolide B [(+)-**4**] to a fused carbon connected to O1 between A-D ring (C8b in the full structure). Due to the 1,3-diaxial orientation of the C2 $\beta$ -methyl and the C4 $\beta$ -ester group in the MTPA ester of (+)-**1**, the former methyl group was very strongly influenced by the ester ( $\Delta\delta=-240$  in comparison to -16 measured for the C11 $\alpha$ -methyl).

The C4 absolute stereochemistry of the chromanol ring in (+)-pseudocalanolide C [(+)-**7**] was also determined to be *R* using the modified Mosher's method, thus, establishing the absolute configuration as (2*S*, 3*S*, 4*R*) (6*S*, 7*S*, 8*R* in the full structure).<sup>7</sup> There was essentially no difference in the coupling



constants of 2-H, 3-H, or 4-H, suggesting that the ring conformation was unchanged by esterification. The stereochemistry of the related calanolides could therefore also be deduced by analogy with the results obtained by the modified Mosher's method.

On the other hand the absolute configuration of (+)-inophyllum A [(+)-**16**] was determined to be 10*R*, 11*R*, 12*S* (2*R*, 3*R*, 4*S* in the simple chromanol ring) from a single crystal X-ray analysis of its 4-bromobenzoate derivative.<sup>5</sup> The stereochemistry of the chromanol ring in all other inophyllums was determined by comparing their NOE enhancements and coupling constants with those of (+)-inophyllum A [(+)-**16**]. (+)-Inophyllum P [(+)-**14**] was originally thought to be soulattrolide [(-)-**14**] until it was determined that (+)-inophyllum P [(+)-**14**] possesses a specific rotation opposite in sign to that published for soulattrolide<sup>28</sup> [(-)-**14**], establishing (+)-inophyllum P [(+)-**14**] as the enantiomer of soulattrolide [(-)-**14**] and the epimer of (+)-inophyllum B [(+)-**13**].

Meinwald and co-workers precisely examined the absolute stereochemistry of soulattrolide [(-)-inophyllum P] [(-)-**14**] and (-)-cordatolide B [(-)-**37**] by application of the modified MTPA method.<sup>33</sup> A correlation of <sup>1</sup>H NMR chemical shift differences with those predicted by Mosher's concept alone was inadequate to assign confidently the absolute stereochemistries, due to the fact that, in both of these molecules, too few protons are present on one side of the MTPA plane. However, energetically favorable conformations obtained by molecular mechanics calculations provided satisfactory rationalizations for the observed anisotropic shifts in <sup>1</sup>H NMR data. Combining the two techniques led to the assignment of the absolute configurations of both soulattrolide [(-)-inophyllum P] [(-)-**14**] and (-)-cordatolide B [(-)-**37**] as (10*R*, 11*R*, 12*S*). The absolute configurations of the other structurally related coumarins, including calanolides A, B, and C were also assigned on the basis of chemical conversions and correlations of their chiroptical properties.

Meinwald and co-workers<sup>33</sup> and our group<sup>6</sup> pointed out subtleties in the application of the Cahn-Ingold-Prelog rules to the designation of *R* or *S* configurations at some positions in these compounds, making basically trivial errors particularly easy. Thus, the original papers reported that the stereochemistry was 10*R*, 11*S*, 12*S* (2*R*, 3*S*, 4*S* in the chromanol ring) for (+)-inophyllum A<sup>5</sup> [(+)-**16**] and 10*R*, 11*R*, 12*S* (2*R*, 3*R*, 4*S* in the chromanol ring) for (+)-calanolide A<sup>1</sup> [(+)-**1**], in which the C11 chiral center had been errorously designated.

## 6. Anti HIV-1 Activity

Three types of *Calophyllum* coumarins, calanolides, inophyllums, and cordatolides, isolated or prepared in pure form were tested for anti HIV-1 activity. In particular (+)-calanolide A [(+)-**1**] and its related calanolides have been intensively examined as promising non-nucleoside RT inhibitors.

**The Calanolides.** Pure calanolides isolated from *C. lanigerum* var. *austrocoriaceum* by the NCI group were comparatively evaluated for anti-HIV activity.<sup>1</sup> In the primary screening assay (+)-calanolide A [(+)-**1**] provided effective protection against the HIV-1 cytopathic effects [EC<sub>50</sub>=0.1 mM, IC<sub>50</sub>=20 μM, therapeutic index (TI)=200]. Although comparable potency was also observed in the ester derivative, (+)-12-acetoxycalanolide A [(+)-**3**] (EC<sub>50</sub>=2.7 μM, IC<sub>50</sub>=13 μM, TI=5) and the C12 epimer, (+)-calanolide B [(+)-**4**] (EC<sub>50</sub>=0.4 μM, IC<sub>50</sub>=15 μM, TI=37), the 12-methoxy derivative [(+)-**2**] and a chromanol-

coumarin belonging to reverse tetracyclic dipyrano-coumarins (Class II), (+)-pseudocalanolide C [(+)-**7**], showed no detectable antiviral activity and, equally, a chromanone-coumarin, (+)-pseudocalanolide D [(+)-**8**], and a tricyclic coumarin, (+)-calanolide E1 [(+)-**10**], were inactive.

The C10 epimer of (+)-calanolide A, (-)-calanolide F [(-)-**6**], showed a moderate activity ( $EC_{50}=ca. 2.8 \mu M$ ,  $IC_{50}=ca. 13 \mu M$ ,  $TI=4.5$ ), which was comparable to that of (+)-12-acetoxycalanolide A [(+)-**3**].<sup>24</sup>

Further examination using purified bacterial recombinant RT revealed that the calanolides were HIV-1 specific RT inhibitors. Moreover, (+)-calanolide A [(+)-**1**] was found to be active not only against the AZT (azidothymidine)-resistant G-9106 strain of HIV-1 but also against the pyridinone-resistant A17 strain. This is of particular interest since the A-17 virus is highly resistant to previously known HIV-1 specific, non-nucleoside RT inhibitors such as TIBO [(+)-(5*S*)-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-thione] which comprise a structurally diverse but apparently common pharmacologic class. Thus, these workers<sup>1</sup> concluded that the calanolides represent a substantial departure from the known class, therefore providing a novel new anti-HIV chemotype for drug development.

Comparative evaluation of the anti HIV-1 activity of enantiomerically pure calanolides A (**1**) and B (**4**) obtained by chiral HPLC resolution techniques revealed that (+)-calanolide A [(+)-**1**] and (-)-calanolide B (costatolide) [(-)-**4**] were potent inhibitors of HIV-1 ( $EC_{50}=0.2$  and  $0.3 \mu M$ , respectively), but that the corresponding enantiomers were inactive.<sup>32</sup> Therefore the observed activity<sup>1</sup> of (+)-calanolide B [(+)-**4**] is presumably due to the presence of the (-)-enantiomer in the scalemic mixture.

The inactivity of unnatural (-)-calanolide A [(-)-**1**] was also reported using ( $\pm$ )-**1** and resolved optically pure enantiomers *in vitro* against both laboratory strains and clinical isolates of HIV.<sup>34</sup> On the basis of the published biological results related to calanolides and inophyllums (*vide infra*) the authors speculated a structure-activity relationship (SAR) of *Calophyllum* coumarins for anti HIV-1 activity as follows: (i) the *trans* configuration between methyl groups at C10 and C11 and the existence of the hydroxyl group was essential because the maximum anti HIV-1 activity was observed in (+)-calanolide A [(+)-**1**] and costatolide [(-)-calanolide B]<sup>32</sup> [(-)-**4**] in calanolides and (+)-inophyllum B<sup>5</sup> [(+)-**13**] and soulattrolide [(-)-inophyllum P]<sup>25</sup> [(-)-**14**] in inophyllum; (ii) the C12-OH must be in the  $\beta$ -position (*S* configuration) in order to maximum anti HIV-1 activity.<sup>35</sup>

Two groups independently tried structural modifications of calanolides for the SAR investigation. Boyd and coworkers prepared a total 14 analogs by either reduction of the  $\Delta^{7,8}$ -olefinic bonds in (+)-calanolide A [(+)-**1**] and (-)-calanolide B [(-)-**4**] or chemical modifications of the C12 hydroxyl group in (-)-calanolide B [(-)-**4**].<sup>36</sup> These analogues were evaluated using a whole cell cytopathicity assay. While none of the compounds showed activity superior to the two unmodified lead compounds, some structure-activity requirements were apparent from the relative anti HIV potencies of various analogs. A heteroatom is required at C12 for activity and reduction of the  $\Delta^{7,8}$ -double bond conferred only a slight reduction in potency.

On the other hand Zembower and coworkers synthesized a series of racemic structural analogs and evaluated them by the same method.<sup>37</sup> Substitution of the C10 methyl group with a longer chain function such as isopropyl group or introduction of extra methyl group into either the 10 or 11 position led to a

lowering of the activity relative to (±)-calanolide A [(±)-**1**]. Analogs containing a *cis* relationship between the C10 and C11 alkyl moieties were completely devoid of activity. Thus, in these structural modifications none of the compounds tested showed activity superior to (±)-calanolide A [(±)-**1**], too.

**The Inophyllums.** Six inophyllums belonging to the class of tetracyclic dipyrano-coumarins (Class I) were evaluated for anti HIV-1 activity.<sup>5</sup> Among them (+)-inophyllum B [(+)-**13**] and P [(+)-**14**] with a *trans*-2,3-dimethylchromanol ring inhibited HIV RT with IC<sub>50</sub>=38 and 130 nM, respectively, and both were active against HIV-1 in cell culture (IC<sub>50</sub>=1.4 and 1.6 μM).<sup>38</sup> However, closely related (+)-inophyllum D [(+)-**15**] and A [(+)-**16**] possessing a *cis*-2,3-dimethylchromanol ring and (+)-inophyllum C [(+)-**19**] and E [(+)-**20**] possessing a chromanone ring were less active or totally inactive, indicating certain structural requirements in the chromanol ring. Thus, although the stereochemistry of the hydroxy group at C12 is not critical, the stereochemistries at C10 (*R*) and C11 (*S*) are important, as maximum activity is observed when the methyl groups at C10 and C11 of the chromanol ring are *trans* diaxial and the C11 methyl group is  $\alpha$ . The most active compound, (+)-inophyllum B [(+)-**13**], has the same stereochemistry as the most active (+)-calanolide A<sup>1</sup> [(+)-**1**] described.

**The Cordatolides.** (+)-Cordatolide A [(+)-**36**] and (-)-cordatolide B [(-)-**37**] belonging to Class I type cordatolides were re-isolated from *C. cordato-oblongum* for the evaluation of anti HIV-1 activity and found to inhibit HIV-1 RT with IC<sub>50</sub>=12.3 and 19.0 μM, respectively.<sup>39</sup> (+)-Cordatolide A [(+)-**36**] has the same stereochemistry as the most active (+)-calanolide A in calanolides [(+)-**1**] and (+)-inophyllum B [(+)-**13**] in inophyllums. However, the activity of (+)-cordatolide A [(+)-**36**] was lower than the expected one, suggesting that the bulkiness of the substituent at C4 is responsible for the activity.

## 7. Conclusion

Thus, (+)-calanolide A [(+)-**1**] in the calanolide series and (+)-inophyllum B [(+)-**13**] in the inophyllum series are the most promising candidates for anti HIV-1 drugs among *Calophyllum* coumarins, although a quite limited amount of the active coumarins is isolable from plant sources. Therefore, it should be necessary to establish their effective synthetic method applicable to asymmetric synthesis. There have been several synthetic trials for them.<sup>40</sup> We have been also approaching to the enantioselective synthesis of *Calophyllum* coumarins based on the intramolecular oxo-Michael type addition reaction of *o*-acryloylphenol derivatives.<sup>41</sup> We hope a non-nucleosidic anti HIV-1 active drug derived from (+)-calanolide A [(+)-**1**] or (+)-inophyllum B [(+)-**13**] could be explored in the near future.

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