# Minor Coumarins from *Calophyllum teysmannii* var. *inophylloide* and Synthesis of Cytotoxic Calanone Derivatives

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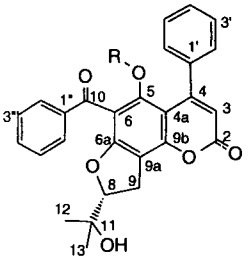
A chemotaxonomic survey for biologically active compounds from Malaysian *Calophyllum* species led to the finding of the four new benzoylcoumarins **1a**, **2**, **3**, and **4a** (including the unusual prenylated 6-benzoylcoumarin **1a**), two uncommon coumarins **5** and **6a** with a pyran-4-one moiety fused at C(6) and C(7), and compounds **7a**, **9**, and **10**, all isolated from the bark of *C. teysmannii* var. *inophylloide*. Their structures were determined by spectroscopic analysis and chemical transformations. X-Ray crystal-structure determination of **2** provided information on the conformational preferences of substituents in this class of coumarins. The syntheses of the cytotoxic calanone (**7a**) and of some related coumarins are described.

**1. Introduction.** – In continuation of our current program concerning the isolation and synthesis of cytotoxic compounds from Malaysian *Calophyllum* species which are rich in triterpenoids [1], biflavonoids [2], xanthonoids [3], and 4-substituted coumarins [4–7], including some 4-alkylcoumarins which are inhibitors of HIV-1 reverse transcriptase [6][7], we have investigated the leaves of *Calophyllum teysmannii* var. *inophylloide*. The tree, found in East Malaysia, was first studied in 1994, and the novel coumarin calanone (**7a**) [8] was isolated. This species is particularly rich in coumarin derivatives, and costatolide, soulattrolide, canalolide F, and other coumarins were subsequently isolated from the latex, bark, leaf and twig [4][5][8–10]. Our chemotaxonomic studies on some of the 46 recorded Malaysian *Calophyllum* species have revealed that there are three varieties of *Calophyllum teysmannii*, namely *C. teysmannii* Miq. var. *teysmannii*, *C. teysmannii* MIQ. var *inophylloide* (King) P. F. Stevens, and *C. teysmannii* MIQ. var. *bursiculum* P. F. STEVENS.

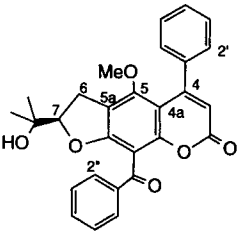
The present study of *C. teysmannii* var. *inophylloide* allowed the isolation of several coumarins including five new minor coumarins (see **1a**, **2**, **3**, **4a**, and **5**) and of other known compounds (see **6a**, **7a**, **9**, and **10**) which have been reported previously [7][8][10][11]. The structures of the new compounds with their cytotoxity data and the syntheses of calanone (**7a**) and related compounds are now presented. Differences in the nature and type of coumarins for *C. teysmannii* var. *inophylloide* are discussed in relation to possible varietal differences among the species.

**2. Structures of Isolated Compounds.** – *Isolation.* The bark material of *C. teysmannii* var. *inophylloide* was exhaustively extracted first with hexane and then with AcOEt. The biologically active (cytotoxic) AcOEt extract was repeatedly chromatographed on silica gel and *Sephadex LH-20* to isolate compounds **1a**, **2**, **3**, **4a**, **5**, **6a**, **7a**, **9**, and **10**[1]).

[1]) Arbitrary numbering of the isoprenyl moiety. For convenience, all coumarins are numbered similarly; for systematic names, see *Exper. Part.*



**1a** R = H[1])
**b** R = Ac[1])



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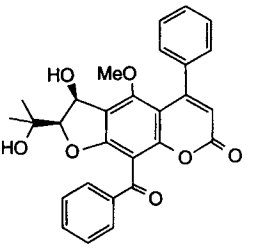

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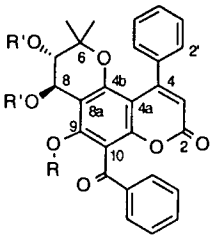
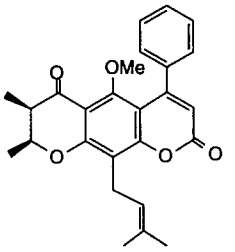


**4a** R = R' = H[1])
**4b** R = Me, R' = H[1])
**4c** R = R' = Ac[1])



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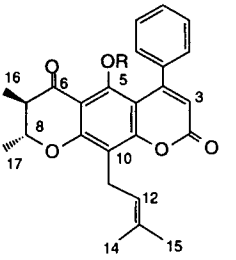
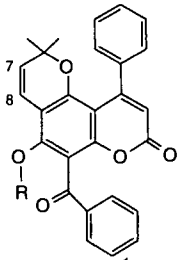
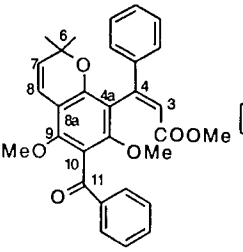


**6a** R = Me[1])
**b** R = H[1])



**7a** R = H[1])
**b** R = Me[1])
**c** R = Ac[1])
**d** R = H,7,8-dihydro[1])



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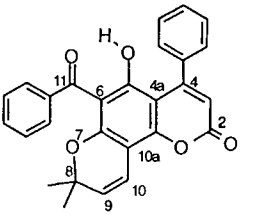

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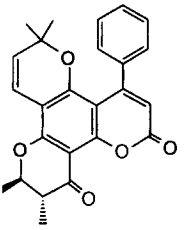

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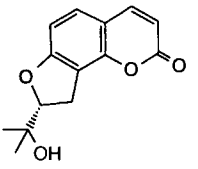
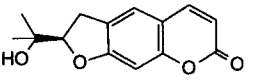


**11**



**12**

*Compound* **1a**. Coumarin **1a** was isolated as a light yellow amorphous powder. Its structure was established by spectroscopic means and transformation to its mono-*O*-acetyl derivative **1b**.

The molecular formula of **1a** was determined as $C_{27}H_{22}O_6$ by HR-MS. In the IR spectrum, absorption bands attributable to an OH (3446 $cm^{-1}$) and two C=O groups (1745 and 1617 $cm^{-1}$ (chelated to OH)) were present. The UV spectrum exhibited absorption maxima at 238, 296, and 348 nm. Extensive analysis of the $^1$H- and $^{13}$C-NMR spectra (*Tables 1* and *2*), together with the use of the HMQC and HMBC spectra, indicated that **1a** was a coumarin with a chelated OH, a Ph, a benzoyl, and an isoprenyl group. In the HMBC spectrum, the proton *s* at $\delta$ 5.99, a typical value for H–C(3) of the 4-phenylcoumarin moiety and which correlated to the C-atom at $\delta$ 112.4 in the HMQC spectrum, showed long-range correlations to C-signals at 160.0 (C(2)), 102.5 (C(4a)), and 139.0 (C(1′)). The C-atom at 139.0 ppm (C(1′)) was also correlated to the aromatic protons at C(3′) and C(5′), which confirmed that the Ph group was attached at C(4) of the coumarin moiety. The position of the chelating OH at group C(5) of **1a** was established by the data of the acetate derivative **1b**, in particular by AcO signal ($\delta$ 1.52) which was strongly shielded by the ring current of the 4-phenyl group lying out of the plane of the coumarin moiety, and by the NOE enhancements at 7.27 and 7.43 ppm (H–C(2′,6′) and H–C(3′,5′)) when the Ac protons were irradiated. Since the $^1$H-NMR spectrum of **1a** showed that HO–C(5) was chelated, the benzoyl group must be substituted *ortho* to it (at C(6)). The isoprenyl $^1$H-NMR signals ($\delta$ 4.57, 3.23, 3.17, 1.10, and 0.94) were compatible with a hydroxydimethylpyran or a 1,2-dihydro-2-(2-hydroxy-2-methylethyl)-furan moiety attached at C(7) and C(8) of an unsubstituted coumarin; the chemical shifts of C(8) and C(11) at $\delta$ 92.8 and 71.3, respectively, established the presence of a 1,2-dihydro-2-(2-hydroxy-2-methylethyl)furan moiety, the $\delta$ of the oxygenated C-atoms of an alternative hydroxy-dimethyl-pyran structure being expected at *ca.* 70 and 80 ppm, respectively [12]. The benzoyl group was not coplanar with the coumarin moiety causing the Me protons of the 2-(2-hydroxy-2-methylethyl) group to be shielded ($\delta$ 0.94 and 1.10). On irradiation of these Me protons (but not of the $CH_2$ protons of the furan ring), NOE enhancements at the signals of $H_{ortho}$ of the benzoyl group were observed.

The negative optical rotation of **1a** ($[\alpha]_D^{25} = -80.5$) may be compared to that of two structurally related coumarins, (−)-columbianelin (**11**) ($[\alpha]_D = -188.3$) and its enantiomer (+)-columbianelin ($[\alpha]_D = +166$) [13]. Based on the known absolute configuration of (−)-columbianelin, (−)-6-benzoyl-5-hydroxy-4-phenylcolumbianelin (**1a**) may tentatively be assigned the same absolute configuration.

*Compound* **2**. Optically active **2** was isolated as needles and its structure determined by comparison of its spectra with those of **1a**.

HR-MS of **2** established the molecular formula $C_{28}H_{24}O_6$. The IR and UV spectra of **2** were very similar to those of **1a**, thus suggesting the structure of an oxygenated coumarin. Also the $^1$H- and $^{13}$C-NMR spectra (*Tables 1* and *2*, resp.) were similar, except for the $^{13}$C-signal of **2** at $\delta$ 59.3 arising from the MeO group. The HMBC spectrum of **2** confirmed the position of the Ph group at C(4). Irradiation of the MeO resonance caused NOE enhancements of both the aromatic protons ($\delta$ 7.30 and 7.40 ppm) of the Ph group and the $CH_2$ protons ($\delta$ 3.23 ppm) of the 2,3-dihydro-2-(2-hydroxy-2-methylethyl)furan moiety. These data showed that the MeO group was attached at C(5) and the 2,3-dihydro-2-(2-hydroxy-2-methylethyl)furan moiety fused at C(6) and C(7) of an unsubstituted coumarin.

Table 1. *$^1$H-NMR Spectral Data for Coumarins* **1a**, **2**, **3**, **4a**, **5**, *and* **6a**[a])[1])

| | **1a** | **2** | **3** | **4a**[a]) | **5** | **6a** |
|---|---|---|---|---|---|---|
| H–C(3) | 5.99 (*s*) | 5.98 (*s*) | 6.00 (*s*) | 5.78 (*s*) | 6.06 (*s*) | 6.07 (*s*) |
| H–C(6) | | 3.31 (*dd*, *J* = 8.1, 15.4)<br>3.23 (*dd*, *J* = 9.4, 15.4) | 5.55 (*d*, *J* = 6.1) | | | |
| H–C(7) | | 4.66 (*dd*, *J* = 8.1, 9.4) | 4.28 (*d*, *J* = 6.1) | 3.57 (*d*, *J* = 7.7) | 2.67 (*dq*, *J* = 3.5, 7.2) | 2.55 (*dq*, *J* = 12.0, 7.1) |
| H–C(8) | 4.57 (*dd*, *J* = 9.1, 9.4) | | | 4.84 (*d*, *J* = 7.7) | 4.69 (*dq*, *J* = 3.5, 6.6) | 4.27 (*dq*, *J* = 12.0, 6.3) |
| H–C(9) | 3.23 (*dd*, *J* = 9.4, 15.5)<br>3.17 (*dd*, *J* = 9.1, 15.5) | | | | | |
| $CH_2$(11)[1]) | | | | | 3.54 (*d*, *J* = 7.5) | 3.54 (*d*, *J* = 7.3) |
| Me(12)[1]) | 1.10 (*s*) | 1.14 (*s*) | 1.48 (*s*) | 1.02 (*s*) | | |
| H–C(12)[1]) | | | | | 5.26 (*tm*, *J* = 7.5) | 5.27 (*tm*, *J* = 7.3) |
| Me(13)[1]) | 0.94 (*s*) | 1.13 (*s*) | 1.27 (*s*) | 0.84 (*s*) | | |
| H–C(14)[1]) | | | | | 1.87 (*s*) | 1.88 (*s*) |
| H–C(15)[1]) | | | | | 1.71 (*s*) | 1.71 (*s*) |
| H–C(16)[1]) | | | | | 1.13 (*d*, *J* = 7.2) | 1.18 (*d*, *J* = 7.1) |
| H–C(17)[1]) | | | | | 1.41 (*d*, *J* = 6.6) | 1.55 (*d*, *J* = 6.3) |
| H–C(2′,6′) | 7.37 (*m*) | 7.30 (*m*) | 7.30 (*m*) | 7.33 (*m*) | | |
| H–C(3′,5′) | 7.43 (*m*) | 7.40 (*m*) | 7.41 | 7.43 (*m*) | 7.32–7.39 (*m*) | 7.30–7.39 (*m*) |
| H–C(4′) | 7.43 (*m*) | 7.40 (*m*) | 7.41 (*m*) | 7.43 (*m*) | | |
| H–C(2″,6″) | 7.53 (*m*) | 7.89 (*dd*, *J* = 7.9, 1.2) | 7.89 (*dd*, *J* = 8.0, 1.2) | 7.88 (*dd*, *J* = 7.4, 1.0) | | |
| H–C(3″,5″) | 7.43 (*m*) | 7.47 (*t*, *J* = 7.9) | 7.47 (*t*, *J* = 8.0) | 7.51 (*t*, *J* = 7.4) | | |
| H–C(4″) | 7.53 (*m*) | 7.59 (*tt*, *J* = 7.9, 1.2) | 7.60 (*tt*, *J* = 8.0, 1.2) | 7.63 (*tt*, *J* = 7.4, 1.0) | | |
| HO–C(5) | 13.08 (*s*) | | | | | |
| MeO–C(5) | | 3.30 (*s*) | 3.60 (*s*) | | 3.10 (*s*) | 3.10 (*s*) |
| HO–C(9) | | | | 12.88 (*s*) | | |

[a]) Measured in $CDCl_3$, except for **4a** ($(D_6)$acetone). Chemical shifts $\delta$ in ppm; coupling constant *J* in Hz.

Table 2. *$^{13}$C-NMR Spectral Data Compounds* **1a**, **2**, **3**, **4a**, **5**, *and* **6a**[a])[1])

| C-number | **1a** | **2** | **3** | **4a** | **5** | **6a** |
|---|---|---|---|---|---|---|
| C(2) | 160.0 (*s*) | 159.4 (*s*) | 159.1 (*s*) | 158.6 (*s*) | 159.5 (*s*) | 159.4 (*s*) |
| C(3) | 112.4 (*d*) | 113.4 (*d*) | 113.5 (*d*) | 111.7 (*d*) | 114.2 (*d*) | 114.0 (*d*) |
| C(4) | 156.2[b]) (*s*) | 155.2[b]) (*s*) | 155.2[b]) (*s*) | 152.3 (*s*) | 156.6 (*s*) | 156.4 (*s*) |
| C(4a) | 102.5 (*s*) | 106.4 (*s*) | 106.0[c]) (*s*) | 102.5 (*s*) | 108.5 (*s*) | 108.3 (*s*) |
| C(4b) | | | | 155.9 (*s*) | | |
| C(5) | 163.5[c]) (*s*) | 154.9[b]) (*s*) | 156.6 (*s*) | | 161.5 (*s*) | 161.6 (*s*) |
| C(5a) | | 114.4 (*s*) | 115.9 (*s*) | | 111.0 (*s*) | 111.5 (*s*) |
| C(6) | 102.9 (*s*) | 28.3 (*t*) | 71.2 (*d*) | 80.2 (*s*) | 193.6 (*s*) | 192.1 (*s*) |
| C(6a) | 163.7[c]) (*s*) | | | | | |
| C(7) | | 91.4 (*d*) | 90.3 (*d*) | 74.0 (*d*) | 46.1 (*d*) | 47.4 (*d*) |
| C(8) | 92.8 (*d*) | | | 68.5 (*d*) | 76.5 (*d*) | 78.7 (*d*) |
| C(8a) | | 161.8 (*s*) | 161.6 (*s*) | 106.4 (*s*) | | |
| C(9) | 27.0 (*t*) | 106.8 (*s*) | 106.2[c]) (*s*) | 156.6 (*s*) | | |
| C(9a) | 105.8 (*s*) | 153.8 (*s*) | 155.6[b]) (*s*) | | 158.6 (*s*) | 158.2 (*s*) |
| C(9b) | 156.3[b]) (*s*) | | | | | |
| C(10) | 199.1 (*s*) | 190.8 (*s*) | 190.9 (*s*) | 108.6 (*s*) | 115.1 (*s*) | 115.0 (*s*) |
| C(10a) | | | | 154.1 (*s*) | 155.6 (*s*) | 155.5 (*s*) |
| C(11) | 71.3 (*s*) | 71.4 (*s*) | 72.8 (*s*) | 193.1 (*s*) | 22.2 (*t*) | 22.0 (*t*) |
| C(12) | 23.9 (*q*) | 25.6 (*q*) | 24.7 (*q*) | 18.6 (*q*) | 120.8 (*t*) | 120.7 (*d*) |
| C(13) | 25.5 (*q*) | 24.5 (*q*) | 28.3 (*q*) | 24.8 (*q*) | 133.1 (*s*) | 132.9 (*s*) |
| C(14) | | | | | 25.9 (*q*) | 25.7 (*q*) |
| C(15) | | | | | 18.1 (*q*) | 17.9 (*q*) |
| C(16) | | | | | 9.2 (*q*) | 10.4 (*q*) |
| C(17) | | | | | 16.0 (*q*) | 19.5 (*q*) |
| C(1′) | 139.0 (*s*) | 139.5 (*s*) | 139.8 (*s*) | 140.6 (*s*) | 139.0 (*s*) | 138.8 (*s*) |
| C(2′,6′) | 127.3 (*d*) | 127.0 (*d*) | 126.9 (*d*) | 127.2 (*d*) | 127.5 (*d*) | 127.4 (*d*) |
| C(3′,5′) | 128.0 (*d*) | 127.6 (*d*) | 128.0 (*d*) | 127.6 (*d*) | 127.6 (*d*) | 127.4 (*d*) |
| C(4′) | 128.4 (*d*) | 128.0 (*d*) | 127.6 (*d*) | 127.2 (*d*) | 128.1 (*d*) | 128.1 (*d*) |
| C(1″) | 140.4 (*s*) | 137.4 (*s*) | 137.6 (*s*) | 138.5 (*s*) | | |
| C(2″,6″) | 127.6 (*d*) | 129.7 (*d*) | 129.7 (*d*) | 129.2 (*d*) | | |
| C(3″,5″) | 127.7 (*d*) | 128.6 (*d*) | 128.6 (*d*) | 128.6 (*d*) | | |
| C(4″) | 131.7 (*d*) | 133.8 (*d*) | 133.8 (*d*) | 133.2 (*d*) | | |
| MeO | | 59.3 (*q*) | 59.4 (*q*) | | 62.5 (*q*) | 62.4 (*q*) |

[a]) Measured in $CDCl_3$, except for **4a** ($(D_6)$acetone). $\delta$ Values in ppm rel. to $SiMe_4$ (= 0 ppm); from DEPT spectra. Assignments are based on NOE-difference, HMQC, and HMBC spectra. [b]) Values in the same column may have interchangeable assignments. [c]) See *Footnote b*.

An X-ray crystal structure of **2** (*Fig.*) confirmed the structural assignments made by NMR and the non-planarity of the Ph and benzoyl groups with respect to the coumarin moiety. Ph–C(4) was significantly out-of-plane, thus causing the shielding of MeO–C(5). The negative optical rotation of **2** ($[\alpha]_D^{25} = -18.1$) was of the same sign and of similar order of magnitude as that of the enantiomer of marmesin ($[\alpha]_D = +26.8$), *i.e.*, nodakenetin (**12**) ($[\alpha]_D = -25.4$) [13]; the latter has the (*R*)-configuration which is also suggested for 9-benzoyl-5-methoxy-4-phenylnodakenetin (**2**).

*Compound* **3**. Optically active **3** was obtained as pale yellow needles and shown to be a 6-hydroxy derivative of **2** by comparison of its spectra.

Compound **3** of molecular formula $C_{28}H_{24}O_7$ (HR-MS) showed IR absorption bands for OH (3420 $cm^{-1}$) and C=O groups (1740 and 1609 $cm^{-1}$) similar to those of **2** and

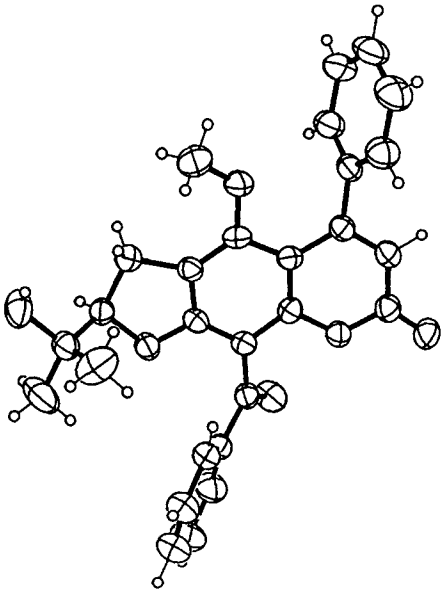


Figure. *A perspective view of molecule* **2**

UV absorption maxima at 254, 294, and 328 nm, typical of an oxygenated coumarin. In the $^1$H-NMR spectrum, ten aromatic protons ($\delta$ 7.30–7.89) and additionally one olefinic proton ($\delta$ 6.00), one MeO group ($\delta$ 3.60), two Me groups ($\delta$ 1.48 and 1.27), and two CH protons ($\delta$ 5.55 and 4.28) belonging to an isoprenyl group were present (*Table 1*). The only difference in the $^{13}$C-NMR spectra of **2** and **3** was that the peak at $\delta$ 28.3 (C(6)) of **2** was replaced by a signal of **3** typical for an oxygenated C-atom at $\delta$ 71.2 (C(6)) (*Table 2*). In the HMBC spectrum, the correlations between C(1′) ($\delta$ 139.8) and H–C(3) ($\delta$ 6.00) and H–C(3′,5′) ($\delta$ 7.41) allowed to position the Ph group at C(4). The 5-position for MeO was confirmed by NOE enhancements of the resonances of H–C(2′,6′) and H–C(6) on saturation of the MeO resonance. The attachment of the isoprenyl group to the coumarin moiety was determined by HMBC long-range correlations (H–C(6) and H–C(7)/C(8a)). According to their coupling constant ($J$ = 6.1 Hz), H–C(6) and H–C(7) were *cis*-arranged [14], and the benzoyl group at C(9) was consistent with the NMR data.

The structure of **3** was thus that of 9-benzoyl-6-hydroxy-5-methoxy-4-phenylnodakenetin, but the absolute configuration was not determined.

*Compound* **4a**. Coumarin **4a** was isolated as a yellow amorphous powder and shown to be a 7,8-dihydroxy derivative of the known calanone (**7a**). Its *O*-methyl and *O*-acetyl derivatives **4b** and **4c**, respectively, confirmed the proposed structure.

Compound **4a** was identified as a 4-phenylcoumarin according to the characteristic H–C(3) resonance at $\delta$ 5.78 which correlated with C(1′), as did protons H–C(3′,5′) in the HMBC spectrum. The $^1$H-NMR spectrum also showed the presence of a benzoyl group and a *trans*-dihydroxy-substituted dimethylpyran moiety ($\delta$ 4.84, 3.57, 1.02, 0.84; $J$(H–C(7), H–C(8)) = 7.7 Hz) (*Table 1*) [15]. On irradiation of the two Me *s* ($\delta$ 0.84 and

1.02) at relatively high fields (shielded by Ph–C(4)), NOE enhancements of H–C(2',6') and H–C(3',5') of the Ph group were observed, suggesting the fusion site of the *trans*-dihydroxy-substituted dimethylpyran moiety at C(5) and C(6) on an unsubstituted coumarin. The EI-MS (HR) failed to give a molecular ion but instead showed an $[M - H_2O]^+$ ion at *m/z* 440.1245 [16]; however, the CI-MS exhibited $M^+$ at *m/z* 458. The Me derivative **4b** gave the required molecular ion for $C_{28}H_{24}O_7^+$. Similarly, the EI-MS of the tri-acetylated product **4c** exhibited a parent ion at *m/z* 584 which further confirmed the molecular formula of **4a** as $C_{27}H_{22}O_7$. In the NOE-difference experiment, irradiation of the MeO group ($\delta$ 3.82) of **4b** caused an enhancement of the methine proton at $\delta$ 4.72 but none for the aromatic protons of Ph–C(4); this verified the assignments of the OH and benzoyl groups of **4a** at the positions shown.

When calanone (**7a**) was epoxidized by 3-chloroperbenzoic acid (*m*-CPBA), the epoxide product was accompanied by the partially hydrolysed dihydroxy derivative which had an identical $R_f$ value on silica gel TLC as the natural product **4a**. The $^1$H- and $^{13}$C-NMR spectra of the prepared 7,8-dihydroxycalanone were also identical to those of the natural product **4a** (*Tables 1* and *2*). Compound **4a** was, therefore, *trans*-7,8-dihydroxycalanone, but the absolute configuration was not determined.

*Compound* **5**. The compound was isolated as an optically active oil of molecular formula $C_{26}H_{26}O_5$ (HR-MS).

The $^1$H-NMR spectrum (*Table 1*) of **5** showed characteristic signals which were assigned to a *cis*-2,3-dimethylchromanone moiety ($\delta$ 2.67 (*dq*, *J* = 3.5, 7.2, H–C(7)); 4.69 (*dq*, *J* = 3.5, 6.6, H–C(8)); 1.13 (*d*, *J* = 7.2, H–C(16)); 1.41 (*d*, *J* = 6.6, H–C(17))) [7], an isoprenyl group, a shielded MeO group ($\delta$ 3.10), an olefinic proton ($\delta$ 6.06), and an unsubstituted Ph group ($\delta$ 7.32–7.39 ppm). A 1-H *s* at 6.06 ppm (H–C(3)) was consistent with a 4-substituted coumarin. Irradiation of H–C(3) and MeO in the NOE-difference experiment caused enhancements of the H–C(2',6') signals of the Ph group, establishing the attachment at C(4) and C(5) of the Ph and MeO group, respectively. The isoprenyl group was determined to be attached at C(8) of an unsubstituted coumarin, and it was also verified that there was no NOE between the MeO and the $CH_2$ group of the isoprenyl moiety.

The structure of compound **5** was assigned to be that of *O*-methylisocalaustralin, but the absolute configuration was not determined.

*Compound* **6a**. Coumarin **6a** was isolated as an optically active oil. The spectral characteristics of compound **6a** indicated that it was isomeric with compound **5**.

UV and IR Spectra of **6a** were very similar to those of compound **5**. HR-MS gave the molecular formula as $C_{26}H_{26}O_5$. The $^1$H-NMR spectrum (*Table 1*) showed the presence of a *trans*-dimethyl-substituted pyran-4-one moiety ($\delta$ 2.55 (*dq*, *J* = 12.0, 7.1, H–C(7)); 4.27 (*dq*, *J* = 12.0, 6.3, H–C(8)); 1.18 (*d*, *J* = 7.1, H–C(16)); 1.55 (*d*, *J* = 6.3, H–C(17))) [7], an isoprenyl chain ($\delta$ 5.27, 3.54, 1.88, and 1.71), a shielded MeO group ($\delta$ 3.10), an olefinic proton ($\delta$ 6.07), and an unsubstituted Ph group ($\delta$ 7.30–7.39 ppm). The positions of the Ph (C(4)) and MeO group (C(5)) were determined by NOE-difference experiments.

To assign the positions of the isoprenyl group and the fusion site of the pyran-4-one moiety, compound **6a** was treated with $BCl_3$ to yield the *O*-demethylated derivative **6b** which showed a chelated OH group ($\delta$ 13.12), indicating that the *trans*-dimethyl-substituted pyran-4-one moiety was fused to the 6,7-positions with the isoprenyl group at C(8)

of an unsubstituted coumarin. The *O*-demethylated product **6b** is the natural product calaustralin which was first isolated by *Beck* and *Stout* [11] from *Calophyllum australianum* and later was also obtained from *C. inophyllum* [17]. Compound **6a**, was, therefore, *O*-methylcalaustralin, but was isolated as a new natural product.

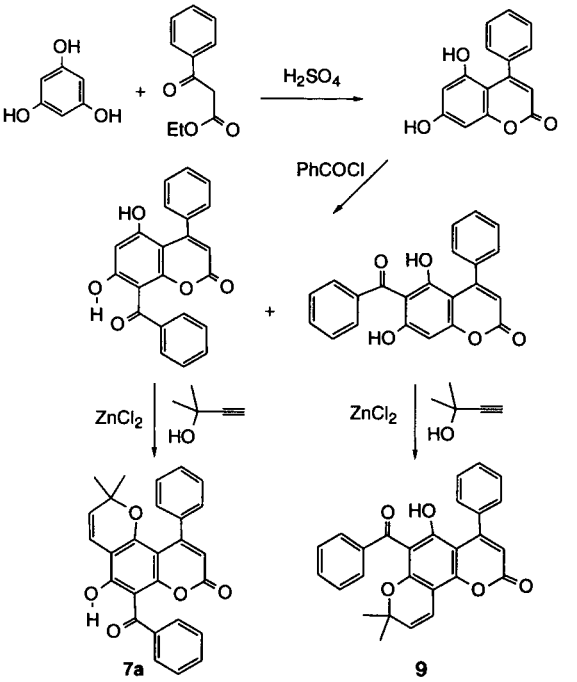
**3. Phytochemical Remarks.** – Although it was known that the genus *Calophyllum* is rich in secondary metabolites including coumarins, the significant number of benzoylated coumarins isolated from the present *Calophyllum* species was unexpected. Only *Calophyllum* plants are presently known to furnish benzoylcoumarins, and it is of chemotaxonomic interest that these compounds are dominant in *C. teysmannii*. Compounds **1a**, **2**, **3**, **4a**, **5**, **6a**, **7a**, **9**, and **10**, among which **7a** and **9** were isolated from the same plant [8][10], all have a parent 4-phenylcoumarin skeleton and are further substituted by benzoyl (**1a**, **2**, **3**, and **4a**) and isoprenyl groups. Compound **1a** from *C. teysmannii* is the first furanocoumarin with a benzoyl group at C(6) of the coumarin moiety, and compound **5** is the first coumarin with a *cis*-2,3-dimethyl-substituted pyran-4-one moiety attached at C(6) and C(7). A previous collection of *C. teysmannii* var. *inophylloide* from Sarawak, the northwestern part of Borneo, provided 4-substituted coumarin derivatives such as soulattrolide and costatolide [8][9] which were not present in the bark of our sample. This may be due to different organs and/or site-specific differences although botanically both specimens were of the same variety out of a possible three varieties of this species.

**4. Synthesis.** – A biomimetic pathway was chosen for the synthesis of compounds **4a**, **7a** [8], and isocalanone (**9**) [10], using phloroglucinol (= benzene-1,3,5-triol) as the starting material (*Scheme 1*). The action of ethyl benzoylacetate on phloroglucinol by the *Pechmann-Duisberg* reaction gave 5,7-dihydroxy-4-phenylcoumarin. Benzoylation of the latter followed by a *Fries* rearrangement provided the 6- and 8-benzoylcoumarins [18]. Finally, the $ZnCl_2$-catalysed electrophilic substitution of the benzoylated 5,7-dihydroxy-4-phenylcoumarins using 2-methylbut-3-yn-2-ol followed by a spontaneous cyclization gave two major products, calanone (**7a**) and isocalanone (**9**) [19] (*Scheme 1*). Calanone (**7a**) was epoxidized with *m*-CPBA to give the 7,8-epoxide, which partially hydrolysed under the reaction and workup conditions to yield racemic **4a**.

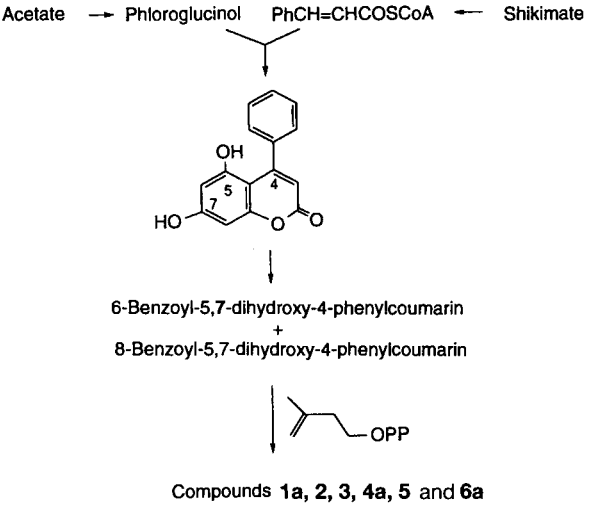
**5. Biogenesis.** – This biosynthesis of these compounds could arise from cinnamic acid or a related derivative and phloroglucinol to form the parent 4-phenylcoumarin backbone, and the subsequent incorporation of benzoyl and isoprenyl groups then would provide the final natural products (*Scheme 2*). The biomimetic synthesis of compounds **4a**, **7a**, and **9** is supportive of the proposed biogenetic origin for these coumarins.

**6. Cytotoxity.** – The present investigation for cytotoxic principles from *C. teysmannii* has led to the finding of five new substituted 4-phenylcoumarins and three known ones. Among the coumarins which had weak-to-moderate cytotoxic activities on four cancer cell lines were coumarins **7a**, **7c**, **8**, and **10** (*Table 3*). Among several of the tested compounds, only **7a** and **10** showed moderate bioactivity. Compound **7a** was more selective to THP-1 cell lines. Inophyllum C (**10**) was, however, more active against THP-1 and MOLT4 cell lines. Among the limited number of compounds tested, no definite relationship was found for the differences in cytotoxic activity, although it appears that

Scheme 1. *Syntheses of* **7a** *and* **9**




Scheme 2. *Biogenetic Origin of Coumarins from* C. teysmannii




the benzoyl group may not be necessary for activity, but a 4-phenyl or 4-propyl substituent seems to be useful. As such compounds are amenable to synthesis, synthetically modified derivatives may provide cytotoxic compounds of greater potential.

Table 3. *Cytotoxicity Bioassay Results* ($ED_{50}$[a])

| | P388 | WEHI164 | THP-1 | MOLT4 |
|---|---|---|---|---|
| **7a** | 26 | 40 | 14.5 | 27.5 |
| **7c** | 24 | 29.5 | 33.5 | 40 |
| **8** | 13.5 | 21 | 42.5 | 30 |
| **10** | 49.5 | 28.5 | 7.8 | 7 |

[a]) $ED_{50}$ values in μg/ml; $ED_{50} < 30$ considered cytotoxic; P388 = mouse leukemia; WEHI1640 = mouse fibrosarcoma; THP-1 = human monocytic leukemia; MOLT4 = lymphoblastic leukemia, human; **7b** and **7d** inactive, $ED_{50} > 40$.

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## Experimental Part

*General.* Liquid chromatography: silica gel *60* (particle size 0.040–0.063 mm) and *Sephadex LH-20*. TLC: silica gel precoated glass plates (*Merck*, silica gel *60* $F_{254}$). M.p.: *Bausch* and *Lamb* hot-stage microscope; uncorrected. UV Spectra: *Hewlett-Packard-8452A* diode array spectrometer, $\lambda_{max}$ in nm. IR Spectra: *Bio-Rad-FTIR* spectrometer; $\tilde{\nu}$ in $cm^{-1}$. NMR Spectra: *Bruker-ACF-300* (300 ($^1$H) and 75 MHz ($^{13}$C)) and *-AMX-500* (500 ($^1$H) and 125 MHz ($^{13}$C)) instruments using $CDCl_3$ or ($D_6$)acetone solns. with $SiMe_4$ as an internal standard, unless otherwise stated; $\delta$ in ppm, $J$ in Hz. EI-MS: *Micromass-VG-7035* mass spectrometer at 70 eV; *m/z* (rel. %).

*Plant Material.* The bark of *Calophyllum teysmannii* var. *inophylloide* was collected from Mt. Tawai, Kinabatangan, Sabah, Malaysia in 1996 and identified by *J. T. Pereira* and *L. Madani*. A voucher specimen (SAN 135177) was deposited at the herbarium of the *Forest Research Centre*, Sepilok, Sandakan, Sabah, Malaysia.

*Extraction and Isolation.* The dried and powdered bark (864 g) of *Calophyllum teysmannii* var. *inophylloide* was extracted first with hexane (24 h, 5 × 6 l), then with AcOEt (24 h, 5 × 6 l), and finally with MeOH (24 h, 5 × 6 l) in a *Soxhlet* apparatus. The AcOEt extract was evaporated to yield a residue (30 g). The residue was fractionated by column chromatography (silica gel *Merck 9385*; 1800 g) eluting with hexane, a gradient of acetone to 100%, followed by $CHCl_3$/MeOH 10:1 → 1:1. The compounds were eluted in the following order: **9** (35 mg, 0.0041%), **7a** (2 g, 0.23%), **5** (1 mg, 0.00012%), **6a** (2 mg, 0.00023%), **10** (5 mg, 0.0006%), **1a** (8 mg, 0.00093%), **2** (38 mg, 0.0044%), **3** (24 mg, 0.0028%) and **4a** (11 mg, 0.0013%).

*(–)-6-Benzoyl-8,9-dihydro-5-hydroxy-8-(1-hydroxy-1-methylethyl)-4-phenyl-2*H*-furo[2,3-*h*]-1-benzopyran-2-one* (**1a**). Pale-yellow amorphous powder. $[\alpha]_D^{25} = -80.5$ ($CHCl_3$, $c = 0.17$). UV (EtOH): 238 (sh), 296, 348. IR (KBr): 3446, 1745, 1617, 1540, 1470, 1389, 1123, 918, 698. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 442 (30 $M^+$), 424 (20), 409 (24), 383 (100). HR-EI-MS: 442.1423 ($M^+$, $C_{27}H_{22}O_6^+$; calc. 442.1416).

*5-(Acetyloxy)-6-benzoyl-8,9-dihydro-8-(1-hydroxy-1-methylethyl)-4-phenyl-2*H*-furo[2,3-*h*][1]benzopyran-2-one* (**1b**). Compound **1a** (2.0 mg) was treated with $Ac_2O$/pyridine 1:1 (0.7 ml) at r.t. for 12 h. Addition of $H_2O$ and extraction with $CHCl_3$ gave a light yellow product which was purified by prep. TLC (silica gel, hexane/AcOEt 3:1): **1b** (2.0 mg, 87%). Amorphous powder. $^1$H-NMR (300 MHz, $CDCl_3$): 1.04 (*s*, Me); 1.15 (*s*, Me); 1.52 (*s*, Ac); 3.38 (*d*, $J = 9.0$, 2 H–C(9)); 4.72 (*t*, $J = 9.0$, H–C(8)); 6.08 (*s*, H–C(3)); 7.27 (*m*, H–C(2′), H–C(6′)); 7.43 (*m*, H–C(3′), H–C(4′), H–C(5′), H–C(3″), H–C(5″)); 7.57 (*m*, H–C(4″)); 7.75 (*m*, H–C(2″), H–C(6″)). EI-MS: 484 (3, $M^+$), 466 (2), 442 (65), 424 (35), 409 (40), 384 (100), 356 (50). HR-EI-MS: 484.1529 ($M^+$, $C_{29}H_{24}O_7^+$; calc. 484.1522).

*(–)-9-Benzoyl-2,3-dihydro-2-(1-hydroxy-1-methylethyl)-4-methoxy-5-phenyl-7*H*-furo[3,2-g][1]benzopyran-7-one* (**2**). Pale yellow needles from EtOH. M.p. 208–210°. $[\alpha]_D^{25} = -18.1$ ($CHCl_3$, $c = 0.77$). UV (EtOH): 224, 252, 292, 336. IR (KBr): 3450, 1733, 1602, 1563, 1470, 1389, 1266, 1115, 953, 691. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 456 (5, $M^+$), 398 (100). HR-EI-MS: 456.1551 ($M^+$, $C_{28}H_{24}O_6^+$; calc. 456.1573).

*(–)-9-Benzoyl-2,3-dihydro-3-hydroxy-2-(1-hydroxy-1-methylethyl)-4-methoxy-5-phenyl-7*H*-furo[3,2-g][1]-benzopyran-7-one* (**3**). Pale yellow needles from EtOH. M.p. 206–208°. $[\alpha]_D^{25} = -8.4$ ($CHCl_3$, $c = 0.47$). UV (EtOH): 254, 294, 328. IR (KBr): 3420, 1740, 1609, 1563, 1470, 1382, 1274, 1123, 1015, 965, 706, 656. $^1$H- and

$^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 472 (5, $M^+$), 454 (4), 439 (4), 397 (100), 368 (50), 319 (40). HR-EI-MS: 472.1514 ($M^+$, $C_{28}H_{24}O_7^+$; calc. 472.1522).

*(−)-6-Benzoyl-3,4-dihydro-3,4,5-trihydroxy-2,2-dimethyl-10-phenyl-2*H,*8*H-*benzo[1,2*-b:*3,4*-b′*]dipyran-8-one* (**4a**). Pale-yellow amorphous powder. $[\alpha]_D^{25} = -31.2$ ($CHCl_3$, $c = 0.22$). UV (EtOH): 252, 324. IR (KBr): 3431, 1722, 1652, 1540, 1455, 1131. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: (100, $[M-18]^+$), 412 (20), 397 (30), 368 (50), 319 (40). CI-MS ($NH_3$): 459 ($[M+1]^+$).

*6-Benzoyl-3,4-dihydro-3,4-dihydroxy-5-methoxy-2,2-dimethyl-10-phenyl-2*H,*8*H-*benzo[1,2*-b:*3,4*-b′*]dipyran-8-one* (**4b**). A mixture of **4a** (2.0 mg), $Me_2SO_4$ (1 ml), and $K_2CO_3$ (0.5 g) in acetone (10 ml) was stirred under $N_2$ at r.t. for 3 h. After workup prep. TLC of the crude product gave **4b** (2.0 mg, 97%). $^1$H-NMR (300 MHz, $CDCl_3$)[1]: 0.88 (*s*, Me); 0.97 (*s*, Me); 2.38 (*d*, *J* = 1.1, OH); 3.60 (*dd*, *J* = 6.6, 1.4, H–C(7)); 3.64 (*d*, *J* = 1.4, OH); 3.82 (*s*, MeO); 4.72 (*dd*, *J* = 6.6, 1.1, H–C(8)); 6.02 (*s*, H–C(3)); 7.23 (*m*, H–C(2′), H–C(6′)); 7.38 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.49 (*m*, H–C(3″), H–C(5″)); 7.63 (*m*, H–C(4″)); 7.93 (*m*, H–C(2″), H–C(6″)). $^{13}$C-NMR (75 MHz, $CDCl_3$)[1]: 18.9 (Me); 24.4 (Me); 62.1 (MeO); 67.5 (C(8)); 74.4 (C(7)); 79.1 (C(6)); 105.4 (C(4a)); 112.4 (C(8a) or C(10) or C(2)); 113.1 (C(10) or C(2) or C(8a)); 114.5 (C(2) or C(8a) or C(10)); 126.9 (C(2′), C(6′)); 127.5 (C(4′)); 127.8 (C(3′), C(5′)); 128.8 (C(3″), C(5″)); 129.7 (C(2″), C(6″)); 134.1 (C(4″)); 137.3 (C(1′)); 139.5 (C(1″)); 151.5 (C(4) or C(4b) or C(10a)); 153.2 (C(4b) or C(10a) or C(4)); 155.1 (C(10a) or C(4) or C(4b)); 158.9 (C(9) or C(2)); 159.2 (C(2) or C(9)); 192.5 (C(11)). EI-MS: 472 (60, $M^+$), 464 (45), 401 (100). HR-EI-MS: 472.1531 ($M^+$, $C_{28}H_{24}O_7^+$; calc. 472.1522).

*6-Benzoyl-3,4-dihydro-2,2-dimethyl-10-phenyl-3,4,5-triacetoxy-2*H,*8*H-*benzo[1,2*-b:*3,4*-b′*]dipyran-8-one* (**4c**). Acetylation of **4a** (2.0 mg) provided a light-yellow amorphous powder (2.2 mg, 86%), after prep. TLC (silica gel, hexane/AcOEt 3:1). $^1$H-NMR (300 MHz, $CDCl_3$)[1]: 0.82 (*s*, Me); 1.06 (*s*, Me); 1.95 (*s*, Ac); 2.03 (*s*, Ac); 2.05 (*s*, Ac); 4.97 (*d*, *J* = 4.0, H–C(7)); 5.90 (*d*, *J* = 4.0, H–C(8)); 6.13 (*s*, H–C(3)); 7.28 (*m*, H–C(2′), H–C(6)); 7.42 (*m*, H–C(3′), H–C(4′), H–C(5′)); 7.48 (*m*, H–C(3″), H–C(5″)); 7.61 (*m*, H–C(4″)); 7.88 (*m*, H–C(2″), H–C(6″)). EI-MS: 584 (5, $M^+$), 542 (4), 524 (5), 500 (1), 482 (94), 440 (70), 422 (100). HR-EI-MS: 584.1688 [$M^+$, $C_{33}H_{28}O_{10}^+$; calc. 584.1682).

cis-*7,8-Dihydro-5-methoxy-7,8-dimethyl-10-(3-methylbut-2-enyl)-4-phenyl-2*H,*6*H-*benzo[1,2*-b:*5,4*-b′*]dipyran-2,6-dione* (= O-*Methylisocalaustralin*; **5**). Optically active oil. $[\alpha]_D^{25} = -10.8$ ($CHCl_3$, $c = 0.02$). UV (EtOH): 232, 272, 324. IR (KBr): 1743, 1695, 1578, 1384, 1327, 1156, 1108, 864, 768, 698. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 418 (86, $M^+$), 403 (80), 363 (70), 349 (80), 319 (90), 307 (80). HR-EI-MS: 418.1763 ($M^+$; $C_{26}H_{26}O_5^+$; calc. 418.1780).

trans-*7,8-Dihydro-5-methoxy-7,8-dimethyl-10-(3-ethylbut-2-enyl)-4-phenyl-2*H,*6*H-*benzo[1,2*-b:*5,4*-b′*]dipyran-2,6-dione* (**6a**). Optically active oil. $[\alpha]_D^{25} = -18$ ($CHCl_3$, $c = 0.04$). UV (EtOH): 232, 268, 324, 346 (sh). IR (KBr): 1741, 1691, 1577, 1460, 1409, 1384, 1324, 1260, 1124, 1039, 904, 860, 769, 702. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. EI-MS: 418 (70, $M^+$), 403 (70), 363 (40), 349 (65), 319 (90), 307 (60). HR-EI-MS: 418.1807 ($M^+$, $C_{26}H_{26}O_5^+$; calc. 418.1780).

trans-*7,8-Dihydro-5-hydroxy-7,8-dimethyl-10-(3-methylbut-2-enyl)-4-phenyl-2*H,*6*H-*benzo[1,2*-b:*5,4*-b′*]dipyran-2,6-dione* (**6b**). To a soln. of **6a** (2.0 mg) in dry $CH_2Cl_2$ (10 ml), $BCl_3$ (0.5 ml) was added at −28°. The mixture was left at r.t. for 3 h and then evaporated. The residue was purified by prep. TLC (hexane/AcOEt 5:1): **6b** (0.8 mg, 41%). $^1$H-NMR (300 MHz, $CDCl_3$)[1]: 1.21 (*d*, *J* = 7.1, Me(16)); 1.56 (*d*, *J* = 6.2, Me(17)); 1.69 (*s*, Me(15)); 1.85 (*s*, Me(14)); 2.64 (*dq*, *J* = 12.2, 7.1, H–C(7)); 3.46 (*d*, *J* = 7.5, $CH_2$(11)); 4.27 (*dq*, *J* = 12.2, 6.2, H–C(8)); 5.25 (*tm*, *J* = 7.5, H–C(12)); 6.00 (*s*, H–C(3)); 7.30–7.41 (*m*, Ph); 13.12 (OH). $^{13}$C-NMR (75 MHz, $CDCl_3$)[1]: 10.0 (C(16)); 18.0 (C(15)); 19.6 (C(17)); 21.6 (C(11)); 25.8 (C(14)); 45.9 (C(7)); 79.1 (C(8)); 102.3 (C(4a) or C(10) or C(5a)); 103.5 (C(10) or C(5a) or C(4a)); 108.8 (C(5a) or C(4a) or C(10)); 113.0 (C(3)); 121.2 (C(12)); 127.3 (C(2′), (C(6′)); 127.7 (C(4′)); 128.4 (C(3′), C(5′)); 132.7 (C(13)); 138.9 (C(1′)); 155.8 (C(4) or C(10a)); 159.1 (C(10a) or C(4)); 159.8 (C(5) or C(9a) or C(2)); 160.5 (C(9a) or C(2) or C(5)); 160.6 (C(2) or C(5) or C(9a)); 200.1 (C(6)). EI-MS: 404 (85, $M^+$), 389 (80), 361 (100), 349 (45), 333 (65), 305 (75). HR-EI-MS: 404.1629 ($M^+$, $C_{25}H_{24}O_5^+$; calc. 404.1624).

*5,7-Dihydroxy-4-phenyl-2*H-*[1]benzopyran-2-one*. To a mixture of benzene-1,3,5-triol (2.52 g) and ethyl benzoylacetate (3.84 g), conc. $H_2SO_4$ soln. (10 ml) was added, and the soln. was stirred at r.t. for 4 days. The mixture was poured over crushed ice and extracted with $CHCl_3$ (50 ml × 5). Evaporation gave a brown solid which after chromatography (silica gel, hexane/AcOEt 10:1 → 1:1) afforded 5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one (3.56 g, 70%). $^1$H-NMR (300 MHz, ($D_6$)acetone): 5.78 (*s*, H–C(3)); 6.29 (*d*, *J* = 2.0 Hz, H–C(6)); 6.37 (*d*, *J* = 2.0, H–C(8)); 7.38 (*s*, Ph). EI-MS: (96, $M^+$), 247 (15), 226 (95), 196 (100).

*6-Benzoyl-5,7-dihydroxy-4-phenyl-2*H-*[1]benzopyran-2-one*. A suspension of 5,7-dihydroxy-4-phenyl-2*H*-[1]-benzopyran-2-one (1.0 g) and $AlCl_3$ (2.6 g) in $CS_2$ (16 ml) and nitrobenzene (10 ml) was stirred for 30 min. Benzoyl chloride (0.56 g) was added, and stirring was continued for one week at r.t., after which the mixture was

poured into ice-water. After evaporation, the pump-dried residue was column chromatographed (silica gel, hexane/AcOEt 20:1 → 1:1) to give 6-benzoyl-5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one (0.30 g, 20%) as crystals from AcOEt. M.p. 244–246°. UV (EtOH): 252, 326. IR (KBr): 3387, 1736, 1700, 1684, 1637, 1617, 1560, 1420, 1093, 938, 824. $^1$H-NMR (300 MHz, ($D_6$)acetone): 5.90 (*s*, H–C(3)); 6.43 (*s*, H–C(8)); 7.43 (*m*, H–C(2'), H–C(3'), H–C(4'), H–C(5'), H–C(6')); 7.47 (*t*, *J* = 7.6, H–C(3''), H–C(5'')); 7.55 (*t*, *J* = 7.6, H–C(4'')); 7.69 (*dd*, *J* = 7.6, 2.0, H–C(2''), H–C(6'')). $^{13}$C-NMR (75 MHz, ($D_6$)acetone): 97.0 (C(8)); 109.1 (C(4a), C(6)); 112.3 (C(6), C(4a)); 113.8 (C(3)); 129.0 (C(2'), C(6')); 129.2 (C(3'), C(5')); 129.4 (C(4')); 129.8 (C(3''), C(5'')); 130.1 (C(2''), C(6'')); 133.6 (C(4'')); 140.8 (C(1')); 141.9 (C(1'')); 157.1 (C(8a) or C(4)); 160.4 (C(4) or C(8a)); 161.7 (C(2)); 163.4 (C(5) or C(7)); 163.5 (C(7) or C(5)); 200.9 (C(9)). EI-MS: 358 (94, $M^+$), 330 (40), 281 (50). HR-EI-MS: 358.0832 ($M^+$, $C_{22}H_{14}O_5^+$; calc. 358.0841).

*8-Benzoyl-5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one*. This compound was the major product (0.50 g, 35%) in the synthesis of 6-benzoyl-5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one (see above). M.p. 254–256°. UV (EtOH): 252, 326. IR (KBr): 3384, 1736, 1700, 1696, 1635, 1616, 1577, 1559, 1415, 1089, 945, 821, 690. $^1$H-NMR (300 MHz, ($D_6$)acetone): 5.81 (*s*, H–C(3)); 6.43 (*s*, H–C(8)); 7.45 (*m*, H–C(2'), H–C(3'), H–C(4'), H–C(5'), H–C(6')); 7.53 (*dd*, *J* = 7.5, 7.5, H–C(3''), H–C(5'')); 7.64 (*t*, *J* = 7.5, H–C(4'')); 7.85 (*td*, *J* = 7.5, 2.0, H–C(2''), H–C(6'')). $^{13}$C-NMR (75 MHz, ($D_6$)acetone)[1]: 100.7 (C(6)); 103.1 (C(4a)); 113.3 (C(2)); 113.8 (C(8)); 129.0 (C(2'), C(3'), C(5'), C(6')); 129.5 (C(4')); 130.0 (C(3''), C(5'')); 130.5 (C(2''), C(6'')); 134.5 (C(4'')); 140.5 (C(1')); 141.3 (C(1'')); 155.6 (C(8a) or C(4)); 157.3 (C(4) or C(8a)); 160.0 (C(5) or C(7) or C(2)); 160.4 (C(7) or C(2) or C(5)); 162.8 (C(2) or C(5) or C(7)); 197.0 (C(9)). EI-MS: 358 (96, $M^+$), 341 (20), 330 (80), 281 (80), 253 (65). HR-EI-MS: 358.0837 ($M^+$, $C_{22}H_{14}O_5^+$; calc. 358.0841).

*6-Benzoyl-5-hydroxy-8,8-dimethyl-4-phenyl-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-2-one* (= *Isocalanone*; **9**). A stirred mixture of 6-benzoyl-5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one (60 mg), 2-methylbut-3-yn-2-ol (56 mg), and $ZnCl_2$ (300 mg) was heated for 30 min at 100° and one further hour at 120°. The mixture was extracted with $CHCl_3$ and AcOH, then the extract was chromatographed (silica gel, hexane/AcOEt 5:1): **9** (7 mg, 10%). $^1$H- and $^{13}$C-NMR: identical to those of the natural product previously isolated from *C. teysmannii* [10].

*6-Benzoyl-5-hydroxy-2,2-dimethyl-10-phenyl-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-8-one* (= *Calanone*; **7a**). As described for **9**, with 8-benzoyl-5,7-dihydroxy-4-phenyl-2*H*-1-benzopyran-2-one (60 mg): **7a** (7.0 mg, 9%). $^1$H- and $^{13}$C-NMR and TLC ($R_f$): identical to those of the isolated natural product [8].

*Preparation of* **4a**. Calanone (**7a**; 20 mg) was epoxidized with an excess of *m*-CPBA (24 mg) in $CH_2Cl_2$ at r.t. for 3 h. The mixture was separated by prep. TLC ($CHCl_3$/MeOH 10:1) to furnish spontaneously hydrolyzed racemic **4a** (6.0 mg, 25%). MS and NMR: identical to those of the isolated natural product (see above).

*6-Benzoyl-5-methoxy-2,2-dimethyl-10-phenyl-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-8-one* (= *O-Methylcalanone*; **7b**). Methylation of **7a** (2.0 mg) was carried out as described for **4b**: **7b** (2.0 mg, 97%). $^1$H-NMR (300 MHz, $CDCl_3$)[1]: 0.99 (*s*, Me); 1.24 (*s*, Me); 3.72 (*s*, MeO); 5.49 (*d*, *J* = 10.5, H–C(7)); 6.00 (*s*, H–C(3)); 6.48 (*d*, *J* = 10.5, H–C(8)); 7.26 (*m*, H–C(2'), H–C(6')); 7.39 (*m*, H–C(3'), H–C(4'), H–C(5')); 7.47 (*m*, H–C(3''), H–C(5'')); 7.58 (*m*, H–C(4'')); 7.91 (*m*, H–C(2''), H–C(6'')). EI-MS: 438 (30, $M^+$), 423 (100), 379 (20). HR-EI-MS: 438.1480 ($M^+$, $C_{28}H_{22}O_5^+$; calc. 438.1467).

*5-(Acetoxy)-6-benzoyl-2,2-dimethyl-10-phenyl-2*H*,8*H*benzo[1,2-b:3,4-b']dipyran-8-one* (= *O-Acetylcalanone*; **7c**). Acetylation of **7a** (2.0 mg) was carried out as described for **1b**: **7b** (2.2 mg, 91%). Amorphous powder. $^1$H-NMR (300 MHz, $CDCl_3$): 1.00 (*s*, 2 Me); 2.05 (*s*, Ac); 5.52 (*d*, *J* = 10.4, H–C(7)); 6.08 (*s*, H–C(3)); 6.17 (*d*, *J* = 10.4, H–C(8)); 7.28 (*m*, H–C(2'), H–C(6')); 7.45 (*m*, H–C(3'), H–C(4'), H–C(5')); 7.60 (*m*, H–C(3''), H–C(5'')); 7.71 (*m*, H–C(4'')); 7.88 (*m*, H–C(2''), H–C(6'')). EI-MS: 466 (5, $M^+$), 424 (38), 409 (100), 331 (45), 105 (30). HR-EI-MS: 466.1520 ($M^+$, $C_{29}H_{22}O_6^+$; calc. 466.1510).

*6-Benzoyl-3,4-dihydro-5-hydroxy-2,2-dimethyl-10-phenyl-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-8-one* (= *7,8-Dihydrocalanone*; **7d**). Calanone (**7a**; 2.0 mg) in anh. MeOH (2 ml) was hydrogenated over 10% Pd/C (1 mg) for 5 h at r.t. Removal of the catalyst by filtration provided a crude product which was purified by prep. TLC (hexane/AcOEt 5:1): 1.0 mg (50%) of **7d**. $^1$H-NMR (300 MHz, $CDCl_3$): 0.89 (*s*, 2 Me); 1.67 (*t*, *J* = 6.8, 2 H–C(7)); 2.68 (*t*, *J* = 6.8, 2 H–C(8)); 5.88 (*s*, H–C(3)); 7.21 (*m*, H–C(2'), H–C(6')); 7.37 (*m*, H–C(3'), H–C(4'), H–C(5')); 7.47 (*t*, *J* = 7.9, H–C(3''), H–C(5'')); 7.57 (*tt*, *J* = 7.9, 1.9, H–C(4'')); 7.66 (*dd*, *J* = 7.9, 1.9, H–C(2''), H–C(6'')); 12.72 (*s*, OH). EI-MS: 426 (80, $M^+$), 411 (20), 383 (50), 371 (100). HR-EI-MS: 426.1469 ($M^+$; $C_{27}H_{22}O_5^+$; calc. 426.1467).

*Methyl 3-(6-Benzoyl-5,7-dimethoxy-2,2-dimethyl-2*H*-[1]benzopyran-8-yl)-3-phenylprop-2-enoate* (**8**). Calanone (**7a**; 2.0 mg) was reacted with an excess $Me_2SO_4$ (5 ml) and $K_2CO_3$ in dry acetone (10 ml) at r.t. for 24 h. The crude product, after removal of solvents was purified by prep. TLC: **8** (1.0 mg, 50%). $^1$H-NMR (300 MHz, $CDCl_3$): 1.03 (*s*, Me); 1.28 (*s*, Me); 3.38 (*s*, MeO); 3.66 (*s*, MeO); 3.71 (*s*, MeO); 5.55 (*d*, *J* = 10.0, H–C(7)); 6.49 (*s*, H–C(3)); 6.54 (*d*, *J* = 10.0, H–C(8)); 7.33 (*m*, H–C(2'), H–C(6')); 7.43 (*m*, H–C(3'), H–C(4'), H–C(5'),

H–C(3″), H–C(5″)); 7.52 (*tt*, $J$ = 8.0, 1.8, H–C(4″)); 7.94 (*dd*, $J$ = 8.0, 1.8, H–C(2″), H–C(6″)). EI-MS: 484 (60, $M^+$), 469 (100), 454 (10), 371 (100). HR-EI-MS: 484.1469 ($M^+$, $C_{30}H_{28}O_6^+$; calc. 484.1467).

*Crystal Structure Data of* **2**. $C_{28}H_{24}O_6$, $M$ 456.47; monoclinic, $P2_1/c$, $a$ = 13.8265(1), $b$ = 9.8159(3), $c$ = 18.1091(6) Å; $\beta$ = 111.594(2)°; $V$ = 2285.3(1) Å$^3$ ($\lambda$ 0.71073 A°), $Z$ = 4, $D_{calc}$ = 1.327 mg/m$^3$, $F(000)$ = 960, $\mu$ = 0.093 mm$^{-1}$, crystal size 0.2 × 0.13 × 0.08 mm$^3$. Frame data were collected at 293(2) K in the range of 2.35–29.21° ($-10 \leq h \leq 18$; $-11 \leq k \leq 12$; $-24 \leq l \leq 24$) on a *Siemens-SMART-CCD* system and processed. The processed *hkl* data were absorption-corrected using the program SADABS. Anisotropic thermal parameters were refined for all the non-H-atoms. All the H-atoms were located in the difference *Fourier* routines. The positional parameter of the OH H-atom was refined. Riding models were used to place the rest of the H-atoms in their idealized positions. In the final least-squares refinement cycles on $F^2$, the model converged at $R_1$ = 0.0626, $wR_2$ = 0.1065, and GOF = 0.991 for 2430 reflections with $F_o > 4\sigma(F_o)$ and 314 parameters. In the final difference *Fourier* synthesis, the electron density fluctuated in the range 0.21 to −0.16 eÅ$^{-3}$. Copies of the crystallographic data (excluding structure factors) are available, free of charge, on application to the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ, UK (fax: +44(1223)336033: e-mail: deposit@ccdc.can.ac.uk) citing the deposition No CCDC-101373.

*Bioassays*. The following cell lines were used: P388 (mouse lymphocytic leukemia), WEHI1640 (mouse fibrosarcoma), THP-1 (human monocytic leukemia), MOLT4 (human lymphoblastic leukemia). Cell survival was evaluated by using the MTT-tetrazolium assay as described previously [20]. Results are given in *Table 3*; according to the criterion set by the National Cancer Institute, USA, $ED_{50}$ values of less than 30 μg/ml are considered cytotoxic [21].

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