

**THREE NEW COUMARINS FROM *CALOPHYLLUM TEYSMANNII*  
VAR. *INOPHYLLOIDE* (GUTTIFERAE)**

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**Abstract** – A chemotaxonomic survey of Malaysian *Calophyllum* plants for potential bioactive compounds provided three new coumarins [12-methoxyinophyllum P (**1**), hydrohydroxyisocalanone (**2**) and 4-phenyl-5-methoxy-7-hydroxy-8-benzoylcoumarin (**3**)] from the bark of *C. teysmannii* var. *inophylloide*, together with known compounds calanone (**4**) and betulinic acid. Their structures were determined by spectroscopic analysis including 2D NMR.

Plants from the *Calophyllum* genus (Guttiferae) are known to be rich sources of triterpenes,<sup>1</sup> xanthenes,<sup>2</sup> biflavonoids,<sup>3</sup> coumarins<sup>4</sup> and neoflavonoids.<sup>5</sup> Since the discovery of the anti-HIV1 activity of calanolide A,<sup>6</sup> inophyllums B and P<sup>7</sup> from some Malaysian *Calophyllum* species significant attention has been directed to these plants.<sup>8-12</sup> Our previous work on the chemical constituents of a related species, *C. gracilipes*, resulted in the isolation of a new xanthone, gracilixanthone,<sup>2</sup> and a novel *seco-trisnor*-triterpenoid, gracilipene.<sup>1</sup> Recently, a collection of the bark of *C. teysmannii* var. *inophylloide*, allowed us to isolate three new coumarins which form the topic of this paper.

The bark of *C. teysmannii* var. *inophylloide* was first extracted with hexane and then with ethyl acetate. The ethyl acetate extract was subjected to column chromatography on silica gel and Sephadex LH-20 to give 12-methoxyinophyllum P (**1**), hydrohydroxyisocalanone (**2**) and 4-phenyl-5-methoxy-7-hydroxy-8-benzoylcoumarin (**3**), together with the known calanone (**4**)<sup>8</sup> and the triterpene betulinic acid.

12-Methoxyinophyllum P (**1**) was obtained as a pale yellow oil,  $[\alpha]_D^{25} +25.5^\circ$  (c 0.5, CHCl<sub>3</sub>), analysed for C<sub>26</sub>H<sub>26</sub>O<sub>5</sub> by HREIMS (found 418.1783, calcd 418.1780). The UV spectrum ( $\lambda_{max}$  232, 278, 286, 334nm) was quite similar to that of inophyllum P.<sup>7</sup> The IR spectrum showed bands which were ascribed to an  $\alpha,\beta$ -unsaturated lactone (1725 cm<sup>-1</sup>) and an unsubstituted phenyl ring (705 and 750 cm<sup>-1</sup>). The <sup>1</sup>H-NMR spectrum showed two methyl singlets ( $\delta$  0.92 and 0.94) and three olefin protons ( $\delta$  6.53, d, J = 10.2 Hz; 5.95, s; 5.34, d, J = 10.2 Hz). Additional proton signals included those of five aromatic protons ( $\delta$  7.40, 3H, m; 7.30, 2H, m) belonging to an unsubstituted phenyl group, a sharp 3H singlet ( $\delta$  3.65, s, -OCH<sub>3</sub>), two methyl doublets ( $\delta$  1.17, d, J = 6.7 Hz; 1.41, d, J = 6.3 Hz), and three methine protons ( $\delta$

1.74, ddq,  $J = 11.0, 2.8, 6.7$  Hz; 4.32, dq,  $J = 11.0, 6.7$  Hz; 4.62, d,  $J = 2.8$  Hz). The  $^1\text{H-NMR}$  spectrum of **1** (see Table 1) was similar to that recorded for inophyllum P<sup>7</sup> but with an additional sharp OMe singlet at  $\delta$  3.65. And the only notable differences between the spectrum of **1** and that reported for inophyllum P were the chemical shift and coupling constants of H-12 (1:  $\delta$  4.62, 1H, d,  $J = 2.8$  Hz; inophyllum P:  $\delta$  5.04, 1H, d,  $J = 3.3$  Hz). This change was brought about by the methoxyl group at position C-12 of **1**.

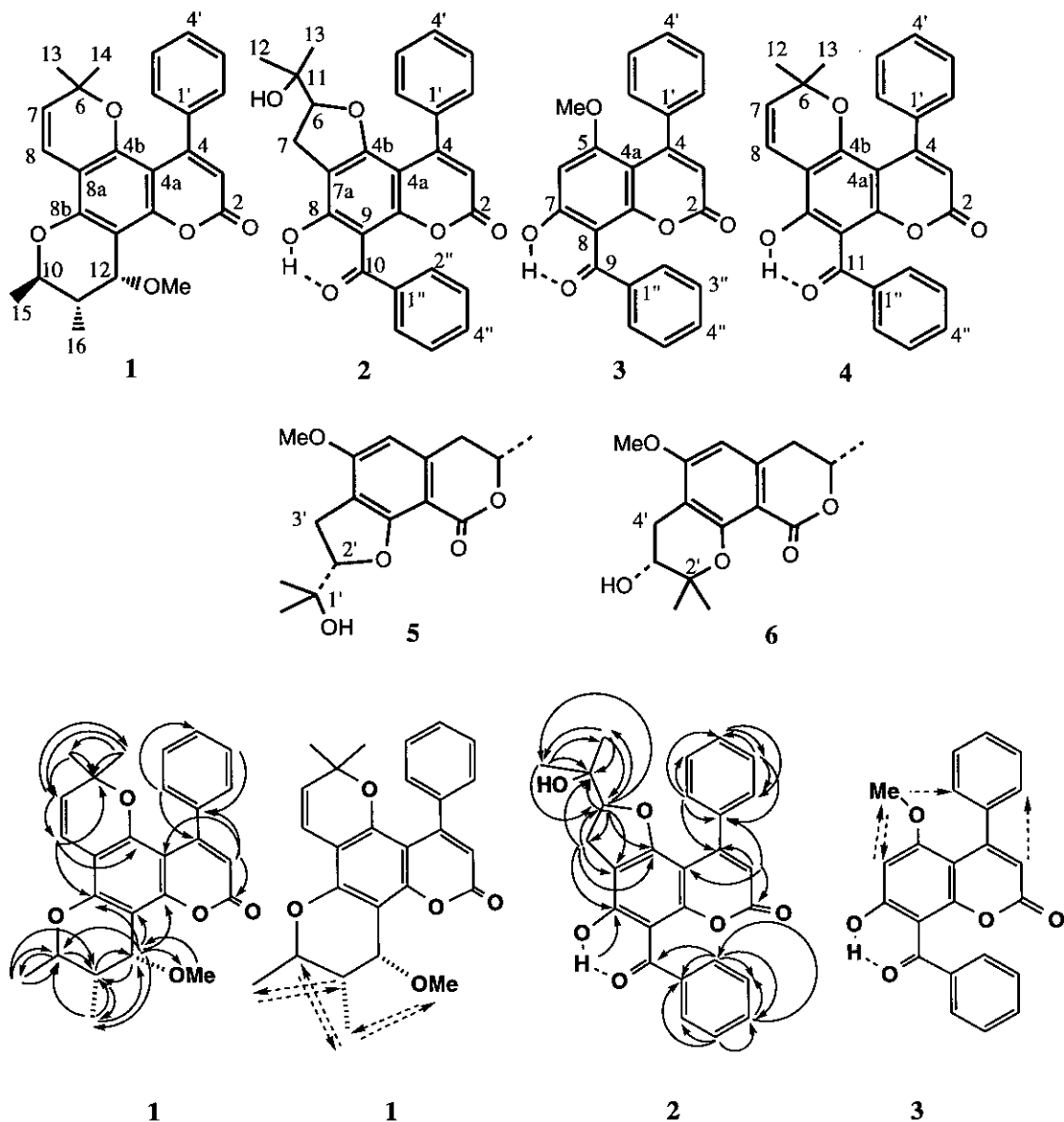


Figure 1 Some HMBC and NOE (---->) Correlations for **1**, **2** and **3**

Table 1  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data for compound (1)

Carbon#	$^{13}\text{C}$ a	$^1\text{H}$ b
2	160.5	
3	111.7	5.95, s
4	156.2	
4a	102.7	
4b	151.2	
6	77.1	
7	127.4	5.34, d, J = 10.2 Hz
8	116.1	6.53, d, J = 10.2 Hz
8a	106.1	
8b	153.5 <sup>c</sup>	
10	73.5	4.32, dq, J = 6.3, 11 Hz
11	38.6	1.74, ddq, J = 2.8, 6.7, 11 Hz
12	70.8	4.62, d, J = 2.8 Hz
12a	104.5	
12b	153.8 <sup>c</sup>	
13	26.88	0.94, s
14	26.93	0.92, s
15	19.1	1.41, 3H, d, J = 6.3 Hz
16	13.3	1.17, 3H, d, J = 6.7 Hz
1'	140.2	
2',6'	127.5	7.30, 2H, m
3',5'	127.4	7.40, 3H, m
4'	127.4	
OCH <sub>3</sub>	59.4	3.65, 3H, s

<sup>a</sup> 125 MHz, CDCl<sub>3</sub>

<sup>b</sup> 500 MHz, CDCl<sub>3</sub>

<sup>c</sup> Resonances may be interchangeable

However the chemical shift and coupling constant of H-12 in **1** matched closely with those of H-12 ( $\delta$  4.54, d, J = 2.5 Hz) in 12-methoxycalanolide B.<sup>6</sup> Except for the methoxyl group, the  $^{13}\text{C}$  NMR spectrum of **1** (see Table 1) was nearly superimposable with that of inophyllum P without considering carbons C-10, C-11, C-12, C-12a, C-15 and C-16 which were all parts of the chromanol ring. The chemical shifts of carbons in the chromanol ring ( $\delta$  73.5, C-10; 38.6, C-11; 70.8, C-12; 104.5, C-12a; 19.1, C-15; 13.3, C-16; 59.4, OCH<sub>3</sub>) were identical with those of 12-methoxycalanolide B<sup>6</sup> ( $\delta$  73.4, C-10; 38.66, C-11; 70.8, C-12; 104.5, C-12a; 19.2, C-15; 13.3, C-16; 59.4, OCH<sub>3</sub>). Based on the above observations, we came to

the conclusion that **1** was the methyl ether of inophyllum P, i.e. 12-methoxyinophyllum P (**1**) [C-10(*R*), C-11(*S*), C-12(*S*)]. And the connectivity and relative stereochemistry of the indicated carbons were confirmed by HMBC and NOE difference experiments (see Figure 1).

Hydrohydroxyisocalanone (**2**) was isolated as an oil in the course of chromatographic separation. The HREIMS molecular ion (found 442.1427, calcd 442.1416) was in accord with the formula  $C_{27}H_{22}O_6$ . The base peak,  $m/z$  424, was also the molecular weight of the known calanone (**4**). The IR and UV spectral properties of the two compounds were similar.<sup>8</sup> The  $^1H$  NMR spectrum of hydrohydroxycalanone (**2**) (see Table 2) was quite similar to that of calanone (**4**) with chemical shift and coupling constant differences arising solely from the hydroxyisopropyl-dihydrofurano moiety of **2** and the 2,2-dimethylchromene system of **4**. The substituents at C-4 and C-8 ( $\delta$  7.66, 2H, dd,  $J = 8.0, 1.5$  Hz; 7.59, 1H, dd,  $J = 8.0, 1.5$  Hz; 7.49, 2H, t,  $J = 8.0$  Hz; 7.44, 3H, m; 7.33, 2H, m) on the coumarin nucleus were seen, by comparison, to be similar to the correspondingly placed substituents in calanone (**4**), i.e. phenyl group at C-4 and benzoyl moiety at C-8 on the coumarin nucleus. A singlet at  $\delta$  5.97 was typical for the C-3 hydrogen of the coumarin nucleus. The low field position of the phenolic proton at  $\delta$  12.18 indicated that it was hydrogen-bonded to the benzoyl group at C-8 of the coumarin nucleus. This ruled out structures in which the rings were linearly fused, because the phenolic hydroxyl would then be situated at C-5 of the coumarin nucleus, unable to chelate with the carbonyl group, and thus would absorb at much higher field. It was apparent, however, that the 2,2-dimethylchromene system present in calanone had been replaced by a new functionality, giving rise to two methyl singlets ( $\delta$  0.96 and 1.05), a single proton signal at  $\delta$  4.56 (dd,  $J = 8.8, 9.8$  Hz), and two one-proton doublet doublet with one centered at  $\delta$  3.01 (dd,  $J = 8.8, 15.5$  Hz) and the other one at  $\delta$  3.14 (dd,  $J = 9.8, 15.5$  Hz). While these absorption could, in principle, be satisfied by either the hydroxyisopropyl-dihydrofurano moiety or by the hydroxydimethylchromanol group, the choice was easily made in favor of the former by comparison of the relevant  $^{13}C$  NMR chemical shifts [ $\delta$  26.9 (C-7), 92.8 (C-6), 71.6 (C-11), 23.1 and 24.9 (C-12 and C-13)] with those of coriandrone A and B [coriandrone A (**5**):  $\delta$  27.23 (C-3'), 92.71 (C-2'), 71.82 (C-1''), 26.23 and 23.58 ( $2 \times Me-1''$ ); coriandrone B (**6**):  $\delta$  26.58 (C-4'), 68.71 (C-3'), 78.24 (C-2'), 24.59 and 22.49 ( $2 \times Me-2'$ )] reported in the literature.<sup>13,14</sup> HMQC and HMBC spectra (see Figure 1) were recorded to confirm the above deductions. There exists a stereochemical centre at C-6 but the isolated compound was racemic; all the  $^1H$  and  $^{13}C$  NMR data (see Table 2) for compound (**2**) could be assigned.

The third new coumarin (**3**) was isolated as yellow needles and had the molecular formula  $C_{23}H_{16}O_5$  by HRMS ( $m/z$  372.10020  $[M]^+$ , calcd 372.0998). The UV spectrum showed  $\lambda_{max}$  252, 290, 328 and the IR spectrum exhibited absorption bands at 3427, 1723, 1627, 739 and 701  $cm^{-1}$ , suggesting the presence of a benzoyl group, a hydroxyl group and a coumarinic lactone, similar to calanone (**4**).<sup>8</sup> The  $^1H$  NMR spectrum (see Table 3) showed signals due to a chelated hydroxyl proton ( $\delta$  12.30, s), an olefinic proton ( $\delta$  5.93, 1H, s), an aromatic proton ( $\delta$  6.33, 1H, s), one methoxyl group ( $\delta$  3.50, 3H, s,  $OCH_3$ ), and ten aromatic protons (phenyl and benzoyl groups). A comparison of **3** with calanone (**4**) showed many similarities in their chemical shifts and coupling constants, e.g. for H-3, the chelated hydroxyl proton, the phenyl and benzoyl protons except for the presence of signals assignable to an aromatic proton ( $\delta$  6.33,

1H, s) and a methoxy group ( $\delta$  3.50, 3H, s, OCH<sub>3</sub>)<sup>15</sup> instead of the signals due to the 2,2-dimethylchromene ring of calanone. NOE correlations confirmed the positions of the methoxyl group ( $\delta$  3.50) and the aromatic proton ( $\delta$  6.33). Irradiation of the aromatic proton ( $\delta$  6.33) showed an NOE enhancement of the methoxyl signal ( $\delta$  3.50). The observed NOE correlation between the methoxyl group ( $\delta$  3.50) and the H<sub>2</sub>-2',6' ( $\delta$  7.24) of the phenyl group confirmed that the methoxyl group was located at C-5 (see Figure 1). Thus, **3** was determined as 4-phenyl-5-methoxy-7-hydroxyl-8-benzoylcoumarin.

Table 2 <sup>13</sup>C and <sup>1</sup>H NMR spectral data for compound (**2**)

Carbon#	<sup>13</sup> C <sup>a</sup>	<sup>1</sup> H <sup>b</sup>
2	158.2	
3	111.9	5.97, s
4	154.2	
4a	98.9	
4b	162.1	
6	92.8	4.56, dd, J = 8.8, 9.8 Hz
7	26.9	3.14, dd, J = 9.8, 15.5 Hz 3.01, dd, J = 8.8, 15.5 Hz
7a	110.0	
8	161.6	
9	104.8	
9a	156.4	
10	198.9	
11	71.6	
12	23.1	0.96, 3H, s
13	24.9	1.05, 3H, s
1'	137.9	
2', 6'	127.6	7.33, 2H, m
3', 5'	128.9	7.44, 2H, m
4'	127.9	7.44, m
1''	140.3	
2'', 6''	128.2	7.66, 2H, dd, J = 8.0, 1.5 Hz
3'', 5''	128.2	7.49, 2H, t, J = 8.0 Hz
4''	132.4	7.59, dd, J = 8.0, 1.5 Hz OH, 12.18, s

<sup>a</sup> 125 MHz, CDCl<sub>3</sub><sup>b</sup> 500 MHz, CDCl<sub>3</sub>

Table 3  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data for compound (3)

Carbon#	$^{13}\text{C}$ a	$^1\text{H}$ b
2	158.1 <sup>c</sup>	
3	112.8	5.93, s
4	155.5 <sup>c</sup>	
4a	102.6 <sup>d</sup>	
5	162.4 <sup>e</sup>	
6	96.1	6.33, s
7	167.0 <sup>e</sup>	
8	104.3 <sup>d</sup>	
8a	156.8 <sup>c</sup>	
9	198.5	
1'	139.5	
2'	126.8	7.24, m
3'	127.4	7.38, m
4'	127.9	7.38, m
5'	127.4	7.38, m
6'	126.8	7.24, m
1''	140.1	
2'',6''	128.1	7.65, 2H, dd, J = 8.0, 1.6 Hz
3'',5''	128.1	7.48, 2H, t, J = 8.0 Hz
4''	132.3	7.60, tt, J = 8.0, 1.6 Hz
		OH, 12.30, s
OCH <sub>3</sub>	55.6	3.50, s

<sup>a</sup> 125 MHz, CDCl<sub>3</sub>

<sup>b</sup> 500 MHz, CDCl<sub>3</sub>

<sup>c,d,e</sup> Resonances may be interchangeable

It is worthy of note that the structures of some of the coumarins above suggest that they have potential as anti-HIV1 protease inhibitors. Previous studies<sup>8-12</sup> have shown that coumarins from *Calophyllum*, e.g. *C. lanigeran* and *C. inophyllum*, which possess appropriate substitution groups at positions 7 and 8 can have potential to provide biologically active compounds.<sup>6,7</sup> In fact, compound (1) which has the substituted dimethylpyran ring is likely to have potential; several related analogues have been targets for synthesis to determine for structure-activity relationships.

## EXPERIMENTAL

*General.* A Reichert-Jung hot-stage microscope was used to measure melting points (uncorrected). EIMS were run on a Micromass VG 7035 mass spectrometer at 70 ev. NMR spectra were recorded by Bruker ACF 300 [300 MHz ( $^1\text{H}$ ) and 75 MHz ( $^{13}\text{C}$ )] and AMX 500 [500 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ )] instruments using  $\text{CDCl}_3$  solutions with TMS as an internal standard unless otherwise stated. IR spectra were recorded on a Bio-Rad FTIR spectrophotometer and UV spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer. Liquid chromatography was performed on silica gel (Kieselgel 60, particle size 0.040-0.063 mm) and Sephadex LH 20. TLC was run on silica gel pre-coated glass plates (Merck silica gel 60 F254).

*Plant material.* The bark of *Calophyllum teysmannii* Miq. *inophylloide* was collected from Sabah, Malaysia in 1995 and identified by J. T. Pereira and L. Madani. A voucher specimen (SAN135177) is deposited at the herbarium of the Forest Research Centre, Sepilok, Sabah, Malaysia.

*Extraction and isolation.* The dried and powdered bark (864 g) of *Calophyllum teysmannii* Miq. *inophylloide* was extracted first with hexane (24 h, 5 x 5L), then with ethyl acetate (24 h, 5 x 5L), and finally with methanol (24 h, 5 x 5L) in a Soxhlet apparatus. The ethyl acetate extract was evaporated to dryness under vacuum to yield a residue (30 g). The residue was fractionated in a silica gel (Merck 9385, 1800 g) column eluted with hexane, and a gradient of acetone was added up to 100%, followed by chloroform:methanol (10:1 to 1:1). The compounds were eluted in the following order: Calanone (**4**)<sup>8</sup> (2 g), 12-methoxyinophyllum P (**1**) (5 mg), 4-phenyl-5-methoxy-7-hydroxy-8-benzoylcoumarin (**3**) (8 mg), hydroxyisocalanone (**2**) (10 mg) and betulinic acid<sup>16</sup> (20 mg).

*Methoxyinophyllum P (1)*: a pale yellow oil;  $[\alpha]_{\text{D}}^{25.5^\circ}$  (c 0.5,  $\text{CHCl}_3$ ); HR-EIMS  $[\text{M}]^+$   $m/z$  418.1783 (calcd for  $\text{C}_{26}\text{H}_{26}\text{O}_5$ , 418.1780); EIMS  $m/z$  (rel. int.) 418  $[\text{M}^+]$  (42), 403 (100), 388 (32), 372 (50), 347 (82); UV  $\lambda_{\text{max}}$  (nm) 232, 278 (sh), 286, 334; IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ) 2983, 2914, 1725, 1630, 1570, 1560, 1455, 1382, 1127, 857, 750, 705, 683; the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data are listed in Table 1.

*Hydroxyisocalanone (2)*: a yellow oil;  $[\alpha]_{\text{D}}^{0^\circ}$  (c 1.0,  $\text{CHCl}_3$ ); HR-EIMS  $[\text{M}^+]$  442.1427 (calcd for  $\text{C}_{27}\text{H}_{27}\text{O}_6$ , 442.1416); EIMS  $m/z$  (rel. int.) 442  $[\text{M}^+]$  (40), 424 (6), 409 (30), 383 (83), 105 (100), 77 (77); UV  $\lambda_{\text{max}}$  (nm) 236, 256, 318; IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ) 3454, 3061, 2925, 2854, 1739, 1603, 1446, 1382, 1283, 1140, 854, 768, 698, 606; the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data are listed in Table 2.

*4-Phenyl-5-methoxy-7-hydroxy-8-benzoylcoumarin (3)*: yellow needles from  $\text{CHCl}_3$ , mp 220-222  $^\circ\text{C}$ ; HR-EIMS  $m/z$  372.1002 (calcd for  $\text{C}_{23}\text{H}_{16}\text{O}_5$ , 372.0998); EIMS  $m/z$  (rel. int.): 372 ( $\text{M}^+$ , 62), 371 (100), 343 (40), 149 (6), 105 (54), 77 (57); UV  $\lambda_{\text{max}}$  (nm): 252, 290 (sh), 328; IR  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3427, 3082, 2927, 1723, 1627, 1595, 1575, 1360, 1263, 1106, 861, 777, 739, 701; the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data are listed in Table 3.

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