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,[1] xanthones,[2] biflavonoids,[3] coumarins[4] and neoflavonoids.[5] Since the discovery of the anti-HIV1 activity of calanolide A,[6] inophyllums B and P[7] from some Malaysian *Calophyllum* species significant attention has been directed to these plants.[8-12] Our previous work on the chemical constituents of a related species, *C. gracilipes*, resulted in the isolation of a new xanthone, gracilixanthone,[2] and a novel *seco-trisnor*-triterpenoid, gracilipene.[1] 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**)[8] and the triterpene betulinic acid.

12-Methoxyinophyllum P (**1**) was obtained as a pale yellow oil, $[\alpha]_D$ +25.5° (c 0.5, $CHCl_3$), analysed for $C_{26}H_{26}O_5$ by HREIMS (found 418.1783, calcd 418.1780). The UV spectrum (λ_{max} 232, 278, 286, 334nm) was quite similar to that of inophyllum P.[7] The IR spectrum showed bands which were ascribed to an α,β–unsaturated lactone (1725 cm^{-1}) and an unsubstituted phenyl ring (705 and 750 cm^{-1}). The ^{1}H-NMR spectrum showed two methyl singlets (δ 0.92 and 0.94) and three olefin protons (δ 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 (δ 7.40, 3H, m; 7.30, 2H, m) belonging to an unsubstituted phenyl group, a sharp 3H singlet (δ 3.65, s, $-OCH_3$), two methyl doublets (δ 1.17, d, J = 6.7 Hz; 1.41, d, J = 6.3 Hz), and three methine protons (δ

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}H-NMR spectrum of **1** (see Table 1) was similar to that recorded for inophyllum P[7] but with an additional sharp OMe singlet at δ 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**: δ 4.62, 1H, d, J = 2.8 Hz; inophyllum P: δ 5.04, 1H, d, J = 3.3 Hz). This change was brought about by the methoxyl group at position C-12 of **1**.

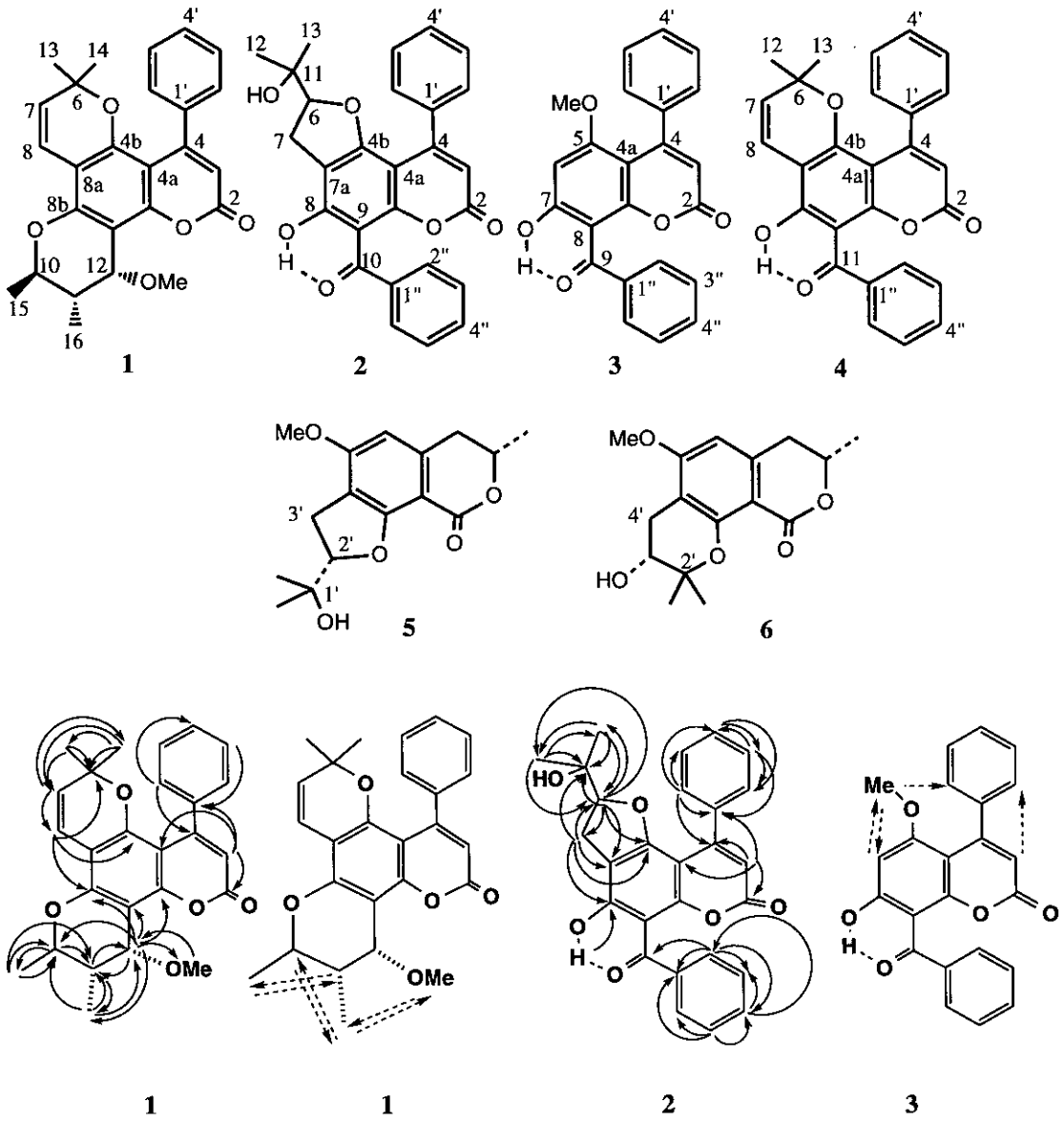

Figure 1 Some HMBC and NOE (⋯→) Correlations for **1**, **2** and **3**

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

Carbon#	^{13}C [a]	^{1}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[c]	
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[c]	
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_3	59.4	3.65, 3H, s

[a] 125 MHz, $CDCl_3$
[b] 500 MHz, $CDCl_3$
[c] Resonances may be interchangeable

However the chemical shift and coupling constant of H-12 in **1** matched closely with those of H-12 (δ 4.54, d, J = 2.5 Hz) in 12-methoxycalanolide B.[6] Except for the methoxyl group, the ^{13}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 (δ 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_3) were identical with those of 12-methoxycalanolide B[6] (δ 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_3). 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.[8] The ^{1}H 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 (δ 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 δ 5.97 was typical for the C-3 hydrogen of the coumarin nucleus. The low field position of the phenolic proton at δ 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 (δ 0.96 and 1.05), a single proton signal at δ 4.56 (dd, J = 8.8, 9.8 Hz), and two one-proton doublet doublet with one centered at δ 3.01 (dd, J = 8.8, 15.5 Hz) and the other one at δ 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 [δ 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**): δ 27.23 (C-3'), 92.71 (C-2'), 71.82 (C-1"), 26.23 and 23.58 (2 × Me-1"); coriandrone B (**6**): δ 26.58 (C-4'), 68.71 (C-3'), 78.24 (C-2'), 24.59 and 22.49 (2 × Me-2')] reported in the literature.[13,14] 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 ^{1}H 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 λ_{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**).[8] The ^{1}H NMR spectrum (see Table 3) showed signals due to a chelated hydroxyl proton (δ 12.30, s), an olefinic proton (δ 5.93, 1H, s), an aromatic proton (δ 6.33, 1H, s), one methoxyl group (δ 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 (δ 6.33,

1H, s) and a methoxy group (δ 3.50, 3H, s, OCH_3)[15] instead of the signals due to the 2,2-dimethylchromene ring of calanone. NOE correlations confirmed the positions of the methoxyl group (δ 3.50) and the aromatic proton (δ 6.33). Irradiation of the aromatic proton (δ 6.33) showed an NOE enhancement of the methoxyl signal (δ 3.50). The observed NOE correlation between the methoxyl group (δ 3.50) and the H_2-2',6' (δ 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 ^{13}C and ^{1}H NMR spectral data for compound (**2**)

Carbon#	^{13}C [a]	^{1}H [b]
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

[a] 125 MHz, $CDCl_3$
[b] 500 MHz, $CDCl_3$

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

Carbon#	^{13}C [a]	^{1}H [b]
2	158.1[c]	
3	112.8	5.93, s
4	155.5[c]	
4a	102.6[d]	
5	162.4[e]	
6	96.1	6.33, s
7	167.0[e]	
8	104.3[d]	
8a	156.8[c]	
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_3	55.6	3.50, s

[a] 125 MHz, $CDCl_3$
[b] 500 MHz, $CDCl_3$
[c,d,e] 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[8-12] 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.[6,7] 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}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using $CDCl_3$ 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 fractioned 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**)[8] (2 g), 12-methoxyinophyllum P (**1**) (5 mg), 4-phenyl-5-methoxy-7-hydroxy-8-benzoylcoumarin (**3**) (8 mg), hydrohydroxyisocalanone (**2**) (10 mg) and betulinic acid[16] (20 mg).

Methoxyinophyllum P (**1**) : a pale yellow oil; $[\alpha]_D$ +25.5° (c 0.5, $CHCl_3$); HR-EIMS $[M]^+$ *m/z* 418.1783 (calcd for $C_{26}H_{26}O_5$, 418.1780); EIMS *m/z* (rel. int.) 418 [M$^+$·] (42), 403 (100), 388 (32), 372 (50), 347 (82); UV λ_{max} (nm) 232, 278 (sh), 286, 334; IR ν_{max} (cm^{-1}) 2983, 2914, 1725, 1630, 1570, 1560, 1455, 1382, 1127, 857, 750, 705, 683; the ^{1}H and ^{13}C NMR spectral data are listed in Table 1.

Hydrohydroxyisocalanone (**2**) : a yellow oil; $[\alpha]_D$ 0° (c 1.0, $CHCl_3$); HR-EIMS [M$^+$] 442.1427 (calcd for $C_{27}H_{27}O_6$, 442.1416); EIMS *m/z* (rel. int.) 442 [M$^+$] (40), 424 (6), 409 (30), 383 (83), 105 (100), 77 (77); UV λ_{max} (nm) 236, 256, 318; IR ν_{max} (cm^{-1}) 3454, 3061, 2925, 2854, 1739, 1603, 1446, 1382, 1283, 1140, 854, 768, 698, 606; the ^{1}H and ^{13}C NMR spectral data are listed in Table 2.

4-Phenyl-5-methoxy-7-hydroxy-8-benzoylcoumarin (**3**) : yellow needles from $CHCl_3$, mp 220-222 °C; HR-EIMS m/z 372.1002 (calcd for $C_{23}H_{16}O_5$, 372.0998); EIMS m/z (rel. int.): 372 (M$^+$, 62), 371 (100), 343 (40), 149 (6), 105 (54), 77 (57); UV λ_{max} (nm): 252, 290 (sh), 328; IR ν_{max} (cm^{-1}) : 3427, 3082, 2927, 1723, 1627, 1595, 1575, 1360, 1263, 1106, 861, 777, 739, 701; the ^{1}H and ^{13}C NMR spectral data are listed in Table 3.

ACKNOWLEDGEMENTS

We thank the National University of Singapore for financial support. Thanks are also due to the Technologists of CIL, Department of Chemistry, NUS, for their running 2D NMR and MS. SGC is grateful to NUS for a scholarship. For plant collections we also thank J. T. Pereira, L. Madani and their assistants of the Forest Research Centre, Sepilok, Sabah, Malaysia.

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Received, 24th June, 1997