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COUMARINS FROM *CALOPHYLLUM TEYSMANNII* (GUTTIFERAE)

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Key Word Index—*Calophyllum teysmannii*; Guttiferae; Teysmanones; Calanone; Inophyllums.

Abstract—From a chemotaxonomic survey of several Malaysian *Calophyllum* species, two new coumarins were isolated from the bark of *Calophyllum teysmannii* Miq. var. *inophylloide* (Guttiferae). The structure elucidation of the new coumarins, teysmanone A (**1**) and teysmanone B (**2**), are presented here and species varieties are discussed. The known calanone (**3**) and inophyllums C and E (**4**, **5**) were also isolated. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The phytochemicals from the genus *Calophyllum* are quite diverse as xanthones [1], flavonoids and biflavonoids [2, 3], neoflavonoids [4], terpenoids [3, 5] and coumarins [6–12] have been found. Recent interest has been focused on several coumarin derivatives which are reported to inhibit the *in vitro* replication and cytopathicity of the human immunodeficiency virus type 1 (HIV-1) [6–12]. In our recent phytochemical surveys of Malaysian flora, we have also examined several *Calophyllum* plants [13, 14]. In Peninsular Malaysia, the genus *Calophyllum* (with as many as 187 species worldwide) is represented by 45 species [15–17]. A taxonomic revision [17] of the Old World species of *Calophyllum* has 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. Our phytochemical collection of *Calophyllum teysmannii* from Sabah showed that the plant is rich in coumarins. Two new coumarin compounds (**1**, **2**) were isolated but three other known compounds (3–5) reported previously [7, 12, 18] were also identified. The new findings were compared to the earlier reports [7, 12, 18] and discussed in relation to possible varietal differences of the species.

RESULTS AND DISCUSSION

The dried and powdered barks of *Calophyllum teysmannii* were successively and exhaustively extracted with hot hexane, ethyl acetate and methanol. TLC investigation indicated the presence of coumarins in both the hexane and ethyl acetate extracts. The chromatographic separation of the ethyl acetate extract furnished (in order of increasing polarity on silica gel) calanone (**3**), teysmanone B (**2**), inophyllum C (**4**), inophyllum E (**5**) and teysmanone A (**1**). Coumarin derivatives (**3–5**), also reported previously [7, 12, 18] from *C. teysmannii* Miq. var. *inophylloide* and from *C. inophyllum*, were identified by comparison of their physical and/or spectral data with those reported.

Coumarin **1** was isolated as fine pale yellow needles from chloroform. The HREI mass spectrum displayed a [M]$^+$ at m/z 424.1340 (calcd 424.13107) indicating a molecular formula of $C_{27}H_{20}O_5$. The UV spectrum (λ_{max} 238, 280 and 334 nm) was quite similar to those of inophyllums [7]. The IR spectrum showed bands which were ascribed to a hydroxyl group (ν_{max} 3423 cm^{-1}), an α,β-unsaturated lactone (ν_{max} 1700 cm^{-1}), unsubstituted phenyl groups (ν_{max} 722 and 691 cm^{-1}), and a conjugated carbonyl (ν_{max} 1671 cm^{-1}). The ^{1}H NMR spectrum of **1** contained a methyl singlet (δ 1.27, 6H), one olefinic proton singlet (δ 5.93), and two doublets (δ 5.55, 1H, *d*, J = 10.2 Hz; δ 6.52, 1H, *d*, J = 10.2 Hz). The remaining signals were found in the aromatic regions (δ 7.91, 2H, *dd*, J = 8.4, 1.2 Hz; δ 7.61, 3H, *m*; δ 7.58, 1H, *dd*, J = 7.4, 1.2 Hz; δ 7.48, 2H, *m*; δ 7.45, 2H, *m*). The ^{13}C NMR spectrum revealed an aromatic ketone (δ 191.9), a conjugated lactone (δ 158.9), a disubstituted olefin (δ 129.1, 1H; δ 115.4, 1H) which is part of a dimethyl-(δ 28.0, 2xMe)-pyran ring, an olefin (δ 113.1, 1H) conjugated to a lactone carbonyl (δ 158.9), a fully substituted benzene ring bearing three oxygen moieties (δ 155.0, 152.7, 149.9, 110.4, 106.3, 101.1), and two unsubstituted phenyl groups (δ 137.6, 135.9, 133.5, 130.6, 130.1 [2xC],

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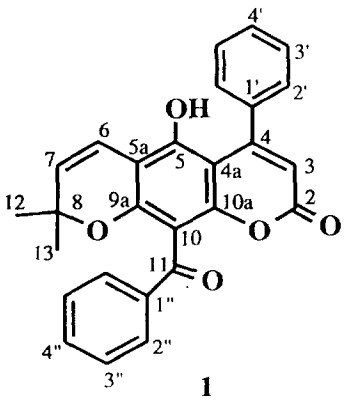

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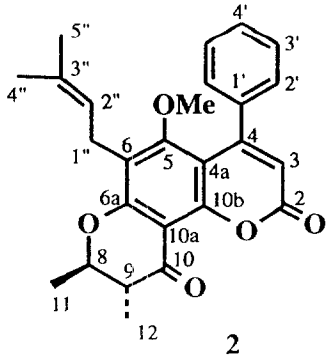

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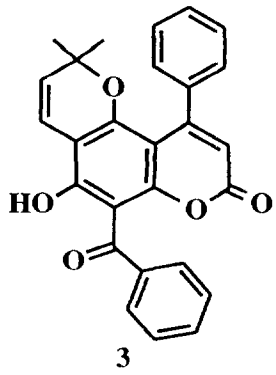

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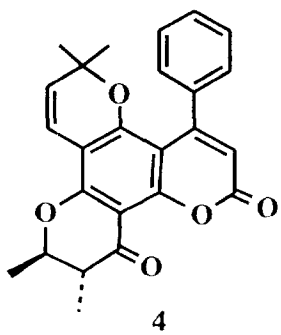

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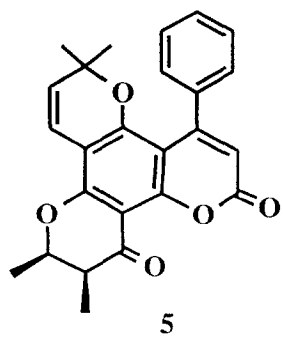

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129.6 [2xC], 128.5 [2xC], 127.5 [2xC]). These data suggested that **1** was a coumarin derivative with phenyl, benzoyl and prenyl groups. Comparison of the HREI mass, ^{1}H NMR and ^{13}C NMR spectra of **1** with those recorded for calanone **3** [12] revealed that **1** was isomeric with calonone. The major difference between these two compounds was that only calanone had a phenolic proton which was hydrogen-bonded and appeared as a sharp singlet at δ 12.46. This meant that the benzoyl group in **1** could be at position 6 or 8 on the coumarin nucleus. Irradiation of the hydroxyl resonance (δ 10.16, 1H, *s*) caused an NOE enhancement of the olefinic proton (δ 6.79, 1H, *d*, J = 10.3 Hz) of the 2,2-dimethylchromene ring (in DMSO-d_6). Methylation and acetylation of teysmanone A (**1**) were carried out to confirm the presence of one phenolic group at C-5 as the derivatives provided upfield-shifted methoxy and acetoxy proton resonances. Irradiation of the methoxy resonance of the methylated derivative of **1** gave rise to NOE enhancement of the olefinic proton at δ 6.49 (1H, *d*, J = 10.4 Hz, H-6). Based on the information given above, we came to the conclusion that **1**, isomeric with calanone, was a coumarin derivative with a phenyl group attached to C-4, a hydroxyl group to C-5, a benzoyl group to C-8, and a linearly fused 2,2-dimethylchromene ring to C-6/C-7 on the coumarin nucleus. HMQC and HMBC spectra were recorded to confirm the above deductions (see Table 1). We were thus able to assign teysmanone A as structure **1**.

Teysmanone B (**2**) was isolated as an optically active oil, $[\alpha]_D$ +40.5°. The molecular ion at *m/z* 418 (EIMS) corresponded to $C_{26}H_{26}O_5$ from high resolution EI mass spectrometry. Its UV spectrum with λ_{max} 226, 286 and 342 nm, similar to inophyllums [7], was characteristic of a coumarin derivative. Its IR spectrum showed the presence of an aromatic carbonyl group and an α,β-unsaturated lactone (ν_{max} 1700 and 1718 cm^{-1}) in addition to bands due to a mono-substituted benzene ring at ν_{max} 780 and 698 cm^{-1}. The ^{1}H NMR spectrum of **2** exhibited two singlets (δ 6.15, 1H; δ 3.00, 3H) and a group of signals belonging to five aromatic protons (δ 7.25–7.43, m). Additional signals included those of a prenyl group (δ 5.08, 1H, *m*; δ 3.29, 2H, *d*, J = 6.9 Hz; δ 1.71, 3H, *s*; δ 1.67, 3H, *s*), and a dimethylchromanone ring (δ 4.33, 1H, *dq*, J = 11.1, 6.5 Hz; δ 2.59, 1H, *dq*, J = 11.1, 7.1 Hz; δ 1.55, 3H, *d*, J = 6.5 Hz; δ 1.24, 3H, *d*, J = 7.1 Hz). The ^{13}C NMR signals observed were in accord with the presence of a coumarin nucleus with the benzenoid ring bearing three oxygen moieties (δ 163.2, 160.4, 159.5, 154.4, 153.7, 120.8, 114.7, 107.2, 106.4), an unsubstituted phenyl ring (δ 138.4, 128.5, 127.7, 127.4), a methoxyl group (δ 62.0), a prenyl group (δ 132.2, 121.7, 25.6, 22.5, 17.8), and a chromanone ring (δ 190.7, 79.5, 47.2, 19.5, 10.4). A comparison of **2** with inophyllum C (**4**) [7] showed considerable similarities of both chemical shifts and coupling constants for the protons of chromanone ring and the unsubstituted phenyl ring, and the signals for H-3 of both compounds had the same chemical shift at δ 6.15 (*s*). These closely matched chemical shifts and coupling

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

Carbon	^{13}C*	^{1}H†	HMBC Correlations
2	158.9		
3	113.1	5.93, *s*	C2, C4a, C1′
4	152.5[a]		
4a	101.1		
5	149.9[b]		
5a	106.3		
6	115.4	6.52, *d*, *J* = 10.2 Hz	C5, C8, C9a
7	129.1	5.55, *d*, *J* = 10.2 Hz	C5a, C8, C12/13
8	78.3		
9a	155.0[b]		
10	110.4		
10a	152.7[a]		
11	191.9		
12	28.0	1.27 (3H), *s*	C7, C8, C13
13	28.0	1.27 (3H), *s*	C7, C8, C12
1′	135.9		
2′, 6′	127.5	7.48 (2H), *m*	C4, C4′, C6′
3′, 5′	130.1	7.61 (2H), *m*	C1′, C5′
4′	130.6	7.61 (1H), *m*	C2′, C6′
1″, 6″	137.6		
2″, 6″	129.6	7.91 (2H), *dd*, *J* = 8.4, 1.2 Hz	C11, C4″, C6″
3″, 5″	128.5	7.45 (2H), *m*	C1″,C5″
4″	133.5	7.58, *dd*, *J* = 7.4, 1.2 Hz	C2″, C6″

*125 MHz, $CDCl_3$.
†500 MHz, $CDCl_3$.
[a,b]Values of resonances interchangeable.

constants for **2** and **4**, especially those on the chromanone ring (δ 4.33, 1H, *dq*, *J* = 11.1, 6.5 Hz; δ 2.59, 1H, *dq*, J = 11.1, 7.1 Hz; δ 1.55, 3H, *d*, *J* = 6.5 Hz; δ 1.24, 3H, *d*, *J* = 7.1 Hz) suggested that they had the same *transoid* stereochemistry. The same sign of rotation (positive) for compounds **2** and **4** indicated that both have configurations C-8*R* and C-9*R*. The compounds differed only in the substituents at C5 and C6 of the coumarin nucleus. The positions of two substituents, methoxyl and prenyl groups, at C5 and C6 on the coumarin nucleus in **2** were confirmed by NOE. An NOE enhancement was observed at δ 3.29 (the methylene protons of the prenyl group) when the methoxyl group at δ 3.00 was irradiated. Normally, the methoxyl protons (at C5 on the coumarin nucleus) are expected to appear at about δ 3.90 [19]. Due to the strong shielding by the ring current of the C4-phenyl ring which is non-coplanar with the coumarin nucleus, the proton NMR absorption of the methoxyl group at C5 of **2** was observed at a relatively high field (δ 3.00). Similar to what was observed for **2**, the methylated and acetylated derivatives of **1** had their methyl absorptions shifted upfield to δ 3.06 and 1.36, respectively. Thus, we assigned the structure **2** to teysmanone B and the spectral data are given in Table 2.

Compound **3**, identified as calanone, was first reported from a study of *Calophyllum teysmannii* var. *inophylloide* [12]. The HREI mass, ^{1}H and ^{13}C NMR spectra obtained matched the reported ones very well with the proton chemical shifts of the two methyls

Table 2. ^{13}C and ^{1}H NMR spectral data for teysmanone 2

Carbon	$\delta^{13}C$*	$\delta^{1}H$†
2	159.5[a]	
3	114.7	6.15, *s*
4	154.4[b]	
4a	107.2[c]	
5	163.2[a]	
6	120.8	
6a	160.4[a]	
8	79.5	4.33, *dq*, *J* = 11.1, 6.5 Hz
9	47.2	2.59, *dq*, *J* = 11.1, 7.1 Hz
10	190.7	
10a	106.4[c]	
10b	153.7[b]	
11	19.5	1.55, *d*, *J* = 6.5 Hz
12	10.4	1.24, *d*, *J* = 7.1 Hz
1′	138.4	
2′, 6′	127.4	
3′, 5′	127.7	7.25–7.43, 5H, m
4′	128.5	
1″	22.6	3.29, 2H, *d*, *J* = 6.9 Hz
2″	121.7	5.08, *m*
3″	132.2	
4″	25.6	1.71, *s*
5″	17.8	1.67, *s*
OCH_3	62.0	3.00, 3H, *s*

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

affected by the ring current of the phenyl group and its structure was unambiguously confirmed by single crystal X-ray diffraction [20]. Compound **4** (inophyllum C) and compound **5** (inophyllum E) were characterized by comparing their spectral data with those reported [7, 18].

The chemical compounds presently isolated bear close resemblance to those reported [8–12] for *Calophyllum teysmannii* var. *inophylloide* although minor compounds **1** and **2** were not reported in previous collections from Sarawak. After closer examination of herbarium samples, it was found that the species investigated in this study was of the same variety, i.e. *C. teysmannii* Miq. var. *inophylloide* (King) P. F. Stevens, which means that differences in the minor phytochemicals were site specific variations. Close similarities among the three different varieties of *C. teysmannii* can be distinguished botanically by a few characters *viz.* terminal bud shape and indumentum; leaf size, texture and indumentum; inflorescence position; pedicel length; and fruit size. In addition, some variations exist in the bark characters and the young plants. Morphologically, var. *teysmannii* and var. *bursiculum* have somewhat conical terminal buds, which initially is enclosed by the petiole bases of the uppermost pair of the leaves, stems often with horizontal lines at the nodes and inflorescences borne in the upper leaf axils. Nevertheless, var. *bursiculum* is distinguished from the rest of the varieties by its prominent V-shaped lines at the nodes of its twigs. On the other hand, var. *inophylloide* has plump terminal buds, which is rarely enclosed by the petioles bases of the uppermost pair of the leaves, stems practically never with horizontal lines at the nodes and the inflorescences often borne in leaf axils along the stem. Our chemical and botanical examination tend to confirm that the Sabah specimen, presently studied, *C. teysmannii* var. *inophylloide*, is similar to the one collected from Sarawak [8–12]. This variety is generally found on well-drained lowland to colline Mixed Dipterocarp Forest, ultramafic soils in Sabah, kerangas vegetation in Sarawak and Brunei and on waterlogged, acid white sands in Kalimantan. The present specimen of *C. teysmannii* var. *inophylloide* has been collected from Mt. Tawai, an ultramafic area in Sabah. It is hoped that if other varieties can be located, a comparison on the phytochemical aspects could be made and further documented.

EXPERIMENTAL

General. Mps: uncorr. UV: EtOH. IR: KBr. ^{1}H NMR: 300 and 500 MHz, ^{13}C NMR: 125 MHz, using TMS as an int. standard. EIMS: 70 eV.

Plant material. Barks of *Calophyllum teysmannii* Miq. var. *inophylloide* were collected from Mt. Tawai, Kinabatangan, Sabah, Malaysia in 1996 and identified by J. T. Pereira and L. Madani. A voucher specimen (SAN135177) was deposited at the Herbarium of the Forest Research Centre, Sabah Forestry Department, Sandakan, Sabah, Malaysia.

Extraction and separation. The dried and powdered bark (864 g) of *Calophyllum teysmannii* were extracted successively with hexane, EtOAc, and MeOH in a Soxhlet apparatus. The hexane fr. afforded the common triterpenes friedelin, friedelanol and stigmasterol. The EtOAc extract was evapd to dryness under vacuum to yield a residue (30 g). The residue was fractioned by silica gel (1800 g) column eluted with hexane, and a gradient of acetone (0 to 100%) was used, followed by $CHCl_3$–MeOH (1:1). The compounds were obtained in the following order: calanone (**3**) (2 g), teysmanone B (**2**) (5 mg), inophyllum C (**4**) (10 mg), inophyllum E (**5**) (5 mg), and teysmanone A (**1**) (30 mg).

Teysmanone A (**1**). pale yellow needles, mp 238–240°; UV λ_{max}^{EtOH} nm (log ε): 238 (4.15), 280 (4.13) and 334 (3.86); IR ν_{max} cm^{-1}: 3423, 1700, 1671, 1598, 1447, 1366, 1247, 1193, 1130, 865, 722, 691; ^{1}H NMR and ^{13}C NMR ($CDCl_3$: see Table 1), ^{1}H NMR (DMSO-d_6): δ 10.16 (1H, *s*, OH), 7.85 (2H, *d*, J = 7.5 Hz), 7.65 (1H, *t*, J = 7.5 Hz), 7.56 (2H, *t*, J = 7.5 Hz), 7.41 (5H, *br s*), 6.79 (1H, *d*, J = 10.3 Hz, H-7), 5.87 (1H, *s*, H-3), 5.69 (1H, *d*, J = 10.3 Hz, H-8), 1.17 (6H, *s*, 2xCH$_3$); EIMS *m/z* (rel. int.): 424 [M]$^+$ (40), 409 (100), 331 (42), 105 (70), 87 (50), 43 (10). HR-EIMS: [M]$^+$ *m/z* 424.1340 ($C_{27}H_{20}O_5$ requires 424.13107).

Acetylation of **1**. Teysmanone A (1 mg) was acetylated at 60° for 24 hr with Ac_2O (0.2 ml) and pyridine (0.5 ml). Removal of excess solvent and reagent and TLC (hexane–EtOAc; 7:1) provided the mono-acetate as a pale yellow powder. ^{1}H NMR (300 MHz) δ 7.93 (2H, *m*), 7.61 (1H, *m*), (3H, *m*), 7.33 (2H, *m*), 7.27 (2H, *m*), 6.14 (1H, *d*, J = 10.4 Hz, H-6), 6.01 (1H, *s*, H-3), 5.68 (1H, *d*, J = 10.4 Hz, H-7), 1.36 (3H, *s*, OAc), and 1.30 (6H, *s*, 2xMe, H-12 and H-13). EIMS *m/z* (rel. int.): 466 [M]$^+$ (10), 451 (20, 424 (6), 409 (100), 331 (22), 105 (46) 77 (38).

Methylation of **1**. Teysmanone A (2 mg) was methylated with methyl iodide (0.1 ml) and K_2CO_3 (10 mg) in Me_2CO (0.5 ml) for 16 hr. Removal of the excess reagent and solvent and prep. TLC (hexane–EtOAc, 7:1) gave the mono-methylated ether. ^{1}H NMR δ 7.92 (2H, *m*), 7.60 (1H, *m*), 7.39–7.49 (7H, *m*), 6.49 (1H, *d*, J = 10.4 Hz, H-6), 6.04 (1H, *s*, H-3), 5.65 (1H, *d*, J = 10.4 Hz, H-7), 3.06 (3H, *s*, OMe), 1.27 (6H, *s*, Mex2, H-12 and H-13). EIMS *m/z* (rel. int.) 438 [M]$^+$ (40), 423 (100), 407 (30), 105 (54), 77 (52).

Teysmanone B (**2**). Yellow oil, $[\alpha]_D$ +40.5° ($CHCl_3$, *c* 0.08); UV λ_{max}^{EtOH} nm (log ε): (3.73), 286 (3.40), 342 (3.22); IR ν_{max} cm^{-1}: 1718, 1700, 1611, 1577, 1463, 1384, 1114, 696; ^{1}H NMR and ^{13}C NMR ($CDCl_3$: see Table 2): EIMS *m/z* (rel. int.) 418[M]$^+$ (100), 403 (70), 387 (10), 363 (25), 349 (60), 335 (35), 319 (80), 307 (40), 105 (42), 55 (45), 43 (70). HR-EIMS [M]$^+$ *m/z* 418.1806 ($C_{26}H_{26}O_5$ requires 418.17801).

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