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COUMARINS FROM *CALOPHYLLUM TEYSMANNII*

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Key Word Index—*Calophyllum teysmannii*; Guttiferae; bark; teysmanones; calanone; inophyllums.

Abstract—During a chemotaxonomic survey of several Malaysian *Calophyllum* species, two new coumarins were isolated from the bark of *Calophyllum teysmannii* var. *inophylloide*. Structure elucidation of the new coumarins, teysmanones A and B are presented and species varieties are discussed. The known calanone and inophyllums C and E were also isolated. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

The phytochemicals from the genus *Calophyllum* are quite diverse and include xanthones [1], flavonoids and biflavonoids [2, 3], neoflavonoids [4], terpenoids [3, Cao *et al.* unpublished] and coumarins [5–11]. Recent interest has been focused on several coumarin derivatives which are reported to inhibit *in vitro* replication and cytopathicity of the human immunodeficiency virus type 1 (HIV-1) [5–11]. In our recent phytochemical surveys of Malaysian flora, we have also examined several *Calophyllum* species [12, 13]. In Peninsular Malaysia, the genus *Calophyllum* (with as many as 187 species worldwide) is represented by 45 species [14–16]. A taxonomic revision [16] of the Old World species of *Calophyllum* has revealed that there are three varieties of *C. teysmannii*, namely *C. teysmannii* Miq. var. *teysmannii*, *C. teysmanii* Miq. var. *inophylloide* (King) P. F. Stevens, and *C. teysmanii* Miq. var. *bursiculum* P. F. Stevens. Our phytochemical collection of *C. teysmannii* from Sabah showed that the plant is rich in coumarins. Two new coumarin compounds (**1** and **2**) were isolated but three other known compounds (**3**–**5**) reported previously [6, 11, 17] were also identified. The new findings were compared with earlier reports [6, 11, 17] and are discussed in relation to possible varietal differences.

RESULTS AND DISCUSSION

Dried and powdered barks of *C. 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. 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 [6, 11, 17] from *C. teysmanii* 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 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 [6]. 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 ^1H NMR spectrum 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, 2 × Me)-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 [2 × C], 129.6 [2 × C], 128.5 [2 × C], 127.5 [2 × C]). These data suggested that **1** was a cou-

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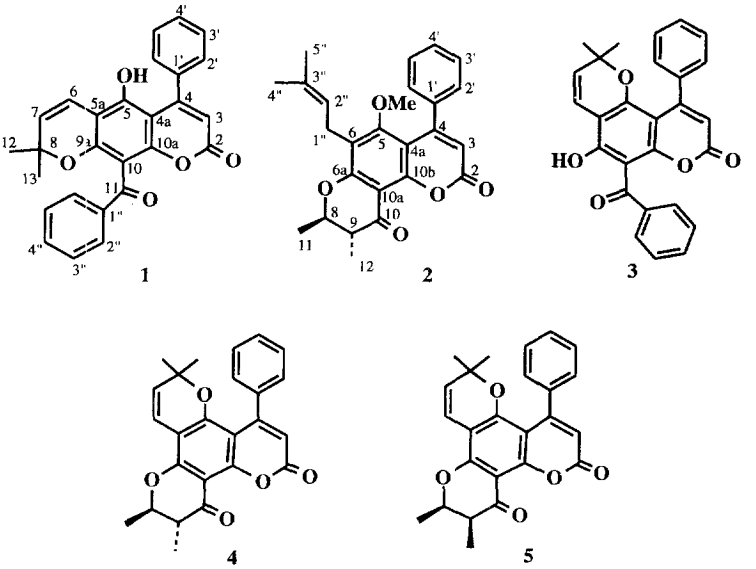

marin derivative with phenyl, benzoyl and prenyl groups. Comparison of the HREI, ^{1}H NMR and ^{13}C NMR spectra of **1** with those recorded for calanone **3** [11] revealed that it was isomeric with calanone. The major difference between these two compounds was that only calanone had a phenolic proton 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 5 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 methoxyl and acetoxyl proton resonances. Irradiation of the methoxyl 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 (Table 1). We were thus able to assign teysmanone A to structure **1**.

Teysmanone B (**2**) was isolated as an optically active oil. The $[M]^+$ at *m/z* (EI) corresponded to $C_{26}H_{26}O_5$ from high resolution measurements. Its UV spectrum with λ_{max} 226, 286 and 342 nm, similar to inophyllums [6], was characteristic of a coumarin derivative. The 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 monosubstituted 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 accordance 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 and 106.4), an unsubstituted phenyl ring (δ 138.4, 128.5, 127.7 and 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 and 10.4). A comparison of **2** with inophyllum C (**4**) [6] showed considerable similarities of both chemical shifts and coupling constants for the protons of the chromanone ring and the unsubstituted phenyl ring; the signals for H-3 of both compounds had the same chemical shift at δ 6.15 (*s*). These closely matched the chemical shifts and coupling 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), suggesting that they had the same *trans*-stereochemistry. The same sign of rotation (positive) for compounds **2** and **4** indicated that both have the configurations C-8*R* and C-9*R*. The compounds differed only in the substituents at C-4 and C-5 of the coumarin nucleus. The positions of two substituents, methoxyl and prenyl groups, at C-4 and C-5 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 3.0 was irradiated. Normally, the methoxyl protons (at C-5 on the coumarin nucleus) are expected to appear at ca δ 3.90 [18]. Due to the strong shielding by the ring

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‡		
4a	101.1		
5	149.9§		
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§		
10	110.4		
10a	152.7‡		
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″	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, $CDCl_3$.
‡,§ Resonances interchangeable.

current of the C4-phenyl ring, which is non-coplanar with the coumarin nucleus, the proton NMR absorption of the methoxyl group at C-5 of **2** was observed at a relatively high field (δ 3.0). 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 structure **2** to teysmanone B and its spectral data are given in Table 2.

Compound **3**, identified as calanone, was first reported from a study of *C. teysmannii* var. *inophylloide* [11]. The HREI, ^{1}H and ^{13}C NMR spectra obtained matched the reported ones, with the proton chemical shifts of the two methyls affected by the ring current of the phenyl group. Its structure was confirmed unambiguously by single crystal X-ray diffraction. Compound **4** (inophyllum C) and compound **5** (inophyllum E) were characterized by comparing their spectral data with those reported [6, 17].

The compounds isolated in the present study closely resemble those reported [7, 11] for *C. teysmannii* var. *inophylloide*, although the 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, 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 bark characteristics and in the young plants. Morphologically, var. *teysmannii* and var. *bursiculum* have somewhat conical terminal buds, which initially are 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 are rarely enclosed by the petiole bases of the uppermost pair of the leaves, stems practically never with horizontal lines at 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, is similar to the one collected from Sarawak [7, 11]. 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 one of the ultramafic areas in Mt. Tawai, Sabah. It is hoped that if

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

Carbon	δ ^{13}C*	δ ^{1}H†
2	159.5‡	
3	114.7	6.15, *s*
4	154.4§	
4a	107.2¶	
5	163.2‡	
6	120.8	
6a	160.4‡	
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¶	
10b	153.7§	
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, $CDCl_3$.
‡,§,¶ Resonances interchangeable.

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 *C. teysmannii* Miq. var. *teysmannii* were collected from 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, Sabah Forestry Department, Sandakan, Sabah, Malaysia.

Extraction and separation

Dried and powdered bark (864 g) 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 fractionated in a silica gel (1800 g) column eluted with hexane and a gradient of Me_2CO was added up to 100%, 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 (EtOH) λ_{max} 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 and ^{13}C NMR ($CDCl_3$: 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, *brs*), 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*, 2 × CH_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 h 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 monoacetate as a pale yellow powder. ^{1}H NMR (300 MHz): δ 7.93 (2H, *m*), 7.61 (1H, *m*), 7.47 (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), 1.30 (6H, *s*, 2 × Me, 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 MeI (0.1 ml) and K_2CO_3 (10 mg) in Me_2CO (0.5 ml) for 16 h. Removal of 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*, Me × 2, 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° ($CHCl_3$, *c* 0.08). UV (EtOH) λ_{max} nm: (log ε): 226 (3.73), 286 (3.40), 3.42 (3.22). IR ν_{max} cm^{-1}: 1718, 1700, 1611, 1577, 1463, 1384, 1114, 696. ^{1}H and ^{13}C NMR ($CDCl_3$: 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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