

Article

Soulamarin, a New Coumarin from Stem Bark of *Calophyllum soulattri*

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Abstract: The extracts of the stem bark of *Calophyllum soulattri* gave a new pyranocoumarin, soulamarin (**1**), together with five other xanthenes caloxanthone B (**2**), caloxanthone C (**3**), macluraxanthone (**4**), trapezifolixanthone (**5**) and brasixanthone B (**6**) one common triterpene, friedelin (**7**), and the steroidal triterpene stigmasterol (**8**). The structures of these compounds were established based on spectral evidence (1D and 2D NMR).

Keywords: soulamarin; pyranocoumarin; *Calophyllum soulattri*; Clusiaceae

1. Introduction

The genus *Calophyllum* comprises over 200 species of trees and shrubs native to tropical Asia, East Africa, India and Australia. *Calophyllum* species have been used in traditional Chinese folk medicine for the treatment of wounds, inflammation and rheumatism. Phytochemical studies on *Calophyllum* species have revealed the presence of xanthenes [1-3], coumarins [4], triterpenoids [5] and flavonoids [6]. The coumarins have been reported to have displayed anti-HIV biological effects [7] and are used as cancer chemo-preventive agents [8]. Various xanthone derivatives show antifungal [9], antimicrobial [10] and molluscicidal [11] effects. We report here the isolation and characterization of a new pyranocoumarin, soulamarin (**1**), from the stem bark of *Calophyllum soulattri*.

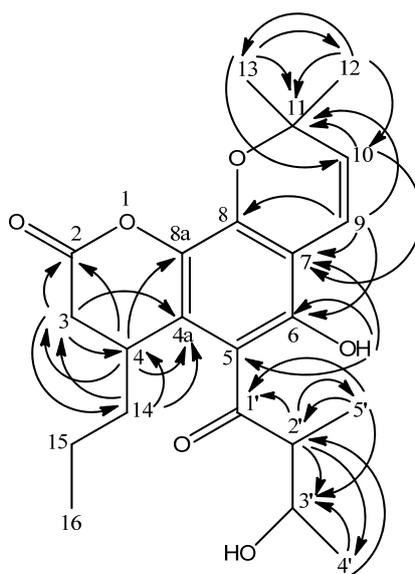
2. Results and Discussion

Soulamarin (**1**) was isolated as yellowish oil from the hexane extract of the stem bark of *Calophyllum soulattri*. The HRESIMS displayed a negative molecular ion peak at m/z 387.1818 $[M-H]^-$ indicating a molecular formula $C_{22}H_{28}O_6$. Compound **1** gave typical coumarin IR absorptions at 3,296 (OH), 2,930 (sp^2 and sp^3 CH), 1,706 (C=O) and 1,621 (C=C) cm^{-1} . Maximum absorptions were observed at 314, 300, 274 and 268 nm in the UV spectrum [12].

The 1H -NMR spectrum revealed the presence of one chelated hydroxyl proton signal at δ 12.46 (*s*, 1H), two vinylic proton signals at δ 6.60 (*d*, 1H, $J = 10.1$ Hz) and 5.46 (*d*, 1H, $J = 10.1$ Hz,) for H-9 and H-10 respectively, three methine signals at δ 2.53 (*m*, H-2'), 4.09 (*m*, H-3') and 3.80 (*m*, H-4) and three methylene signals at δ 1.26 (*m*, H-14), 1.38 (*m*, H-15) and 2.71 and 2.81 (both *dd*, $J = 7.8$ Hz) for H-3a and H-3b. Five methyl signals at δ 1.19 (*d*, $J = 6.4$ Hz), 1.27 (*t*, $J = 3.4$ Hz), 1.42 (*s*), 1.44 (*s*) and 1.49 (*d*, $J = 6.4$ Hz) were also observed. The ^{13}C -NMR spectrum and DEPT experiment showed the presence of seven quaternary carbons at δ 78.2 (C-11), 102.1 (C-5), 102.9 (C-7), 110.9 (C-4a), 157.0 (C-6), 159.6 (C-8) and 159.7 (C-8a), five methines at δ 25.4 (C-4), 45.8 (C-2'), 79.0 (C-3'), 115.8 (C-9), 125.8 (C-10), three methylenes (δ 39.5, 28.4, 29.8 for C-3, C-14 and C-15, respectively) and five methyls (δ 10.4, 19.3, 19.7, 28.3×2 for C-5', C-16, C-4' and C-12 and C-13, respectively). The ^{13}C -NMR spectrum also indicated a carbonyl (δ 179.1) and an aliphatic ketone (δ 199.4).

The structure was further elucidated by HMBC spectral analysis after the assignment of the protons to their direct bonding carbons by the HMQC spectrum. The low field chelated proton at δ 12.46 was due to hydrogen-bonding with the carbonyl group (δ 199.4, C=O) which resulted in the deshielding effect. The 3J and 2J connectivity of the chelated hydroxyl proton at δ 12.46 with δ 102.1 (C-5), 102.9 (C-7) and 157.0 (C-6) confirmed its location at C-6 (see Figure 1).

Figure 1. HMBC 2J and 3J correlations between 1H and ^{13}C in **1**.



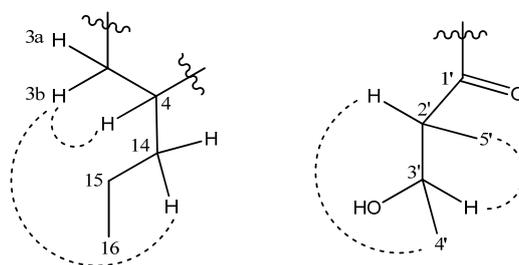
The HMBC experiment also demonstrated long-range 2J and 3J correlations between the two doublet protons at δ 5.46 (H-10, $J = 10.1$ Hz) and 6.60 (H-9, $J = 10.1$ Hz) with the carbon signal at δ 78.2 (C-11) respectively. The linkages between the two aliphatic methyls at δ 1.42 (H-12) and

δ 1.44 (H-13) to the two carbon signals at δ 78.2 (C-11) and 125.8 (C-10) were also seen. These data together with the COSY spectrum analysis suggested the existence of a pyran ring. The pyran ring was clearly fused onto the non-oxygenated carbon C-7 and an oxygenated carbon C-8, as confirmed by the long range (3J) correlations of H-9 (δ 6.60) with C-8 (δ 159.6) and H-10 (δ 5.46) with C-7 (δ 102.9).

Meanwhile, the HMBC correlations of δ 1.19 (H-5') with δ 45.8 (C-2'), 79.0 (C-3') and 199.4 (C-1') proved that these methyl protons were located at position C-2'. The coupling of H-2' and H-3' in the COSY spectrum established the connectivity between δ 45.8 (C-2') and 79.0 (C-3'). The linkage between δ 1.49 (H-4') with δ 45.8 (C-2') and 79.0 (C-3') confirmed its position at C-4'.

An *n*-propyl substituent was found to be attached to C-4 via long range correlations of δ 1.26 (H-14) with δ 25.4 (C-4), 39.5 (C-3) and 110.9 (C-4a). The existence of the *n*-propyl group was also observed in the COSY spectrum through cross peaks between δ 1.26 (H-14) and 1.38 (H-15) and between δ 1.38 (H-15) and 1.27 (H-16). NOESY experiments predicted the relative configurations of soulamarin. Cross peaks were observed between H-3' and H-5' and between H-2' and H-4' suggesting the two proton pairs were oriented on opposite sides. Cross peaks between H-3b and H-4 and between H-3b and H-14 indicate these protons have similar orientations. The NOESY correlations for compound **1** are shown in Figure 2. Taken together, the evidence suggested that compound **1** is 6-hydroxy-4-propyl-5-(3-hydroxy-2-methyl-1-oxobutyl)-6'',6''-dimethylpyrano-[2'',3'':8,7]-benzopyran-2-one (Figure 3).

Figure 2. NOESY correlations between ^1H and ^1H in **1**.



3. Experimental

3.1. Plant Material

The stem bark of *Calophyllum soulattri* was collected from the Sri Aman district in Sarawak, Malaysia. This plant was identified by Dr. Rusea Go from the Department of Biology, Faculty of Science, Universiti Putra Malaysia.

3.2. General

EIMS were recorded on a Shimadzu GC-MS model QP2010 Plus spectrometer. NMR spectra were obtained using a JEOL 500 MHz FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Ultraviolet spectra were recorded in EtOH on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer. Infrared spectra were measured using the universal attenuated total reflection (UATR) technique on a Perkin-Elmer 100 Series FT-IR spectrometer.

3.3. Extraction and Isolation

Approximately 1 kg of air-dried stem bark of *Calophyllum soulattri* was ground to a fine powder and extracted successively in a Soxhlet apparatus with *n*-hexane (68–72 °C, 3 × 2 L) and dichloromethane (40 °C, 3 × 2 L) for 24 hours. The extracts were evaporated to dryness under vacuum to give 101.2 g of *n*-hexane extract and 15.3 g of dichloromethane extract. Part of each extract was subjected to column chromatography over silica gel and eluted with a stepwise gradient system of *n*-hexane, dichloromethane, ethyl acetate and methanol. Further purification of the *n*-hexane extract afforded the new coumarin soulamarin (**1**, 8 mg) and the triterpene friedelin (**7**, 450 mg). Compound **1** was isolated from the *n*-hexane:chloroform (1:4) eluate mixture, followed by several further purifications using a Chromatotron® (Harrison Research) eluting with an *n*-hexane:chloroform (3:2) mixture. *Soulamarin* (**1**): Yellowish Oil; UV (EtOH) λ_{\max} nm: 314, 300, 274, 268; IR ν_{\max} cm^{-1} : 3296, 2930, 1706, 1621; EIMS m/z (rel. int.): 388 [M^+] (9), 373 (53), 360 (42), 345 (100), 301 (14), 285 (13), 245 (9), 229 (24), 55 (11); HRESIMS: 387.1818 [M-H^-] (Calc'd. for $\text{C}_{22}\text{H}_{28}\text{O}_6$: 388.1886); $^1\text{H-NMR}$ (CDCl_3): δ 12.46 (OH-6, s), 6.60 (1H, *d*, $J = 10.1$ Hz, H-9), 5.46 (1H, *d*, $J = 10.1$ Hz, H-10), 4.09 (1H, *m*, H-3'), 3.80 (1H, *m*, H-4), 2.81 (1H, *dd*, $J = 7.8$ Hz, H-3a), 2.71 (1H, *dd*, $J = 7.8$ Hz, H-3b), 2.53 (1H, *m*, H-2'), 1.49 (3H, *d*, $J = 6.4$ Hz, H-4'), 1.44 (3H, *s*, H-13), 1.42 (3H, *s*, H-12), 1.38 (2H, *m*, H-15), 1.27 (3H, *t*, $J = 3.4$ Hz, H-16), 1.26 (2H, *m*, H-14), 1.19 (3H, *d*, $J = 6.4$ Hz, H-5'); $^{13}\text{C-NMR}$ (CDCl_3): δ 199.4 (C-1'), 179.1 (C-2), 159.7 (C-8a), 159.6 (C-8), 157.0 (C-6), 125.8 (C-10), 115.8 (C-9), 110.9 (C-4a), 102.9 (C-7), 102.1 (C-5), 79.0 (C-3'), 78.2 (C-11), 45.8 (C-2'), 39.5 (C-3), 29.8 (C-15), 28.3 × 2 (C-12 & C-13), 25.4 (C-4), 19.7 (C-4'), 19.3 (C-14 & C-16), 10.4 (C-5'). Meanwhile, purification of the dichloromethane extract afforded the five known xanthenes caloxanthone B (**2**, 12 mg), caloxanthone C (**3**, 14 mg), macluraxanthone (**4**, 6 mg), trapezifolixanthone (**5**, 10 mg), brasixanthone B (**6**, 21 mg) and stigmasterol (**8**, 22 mg) (Figure 3).

Caloxanthone B (**2**). Yellow needles; M.P. 157–158 °C (lit. 160.5 °C [13]); Spectral data were consistent with published data [13].

Caloxanthone C (**3**). Yellow needles; M.P. 210–212 °C (lit. 217 °C [14]); Spectral data were consistent with published data [14].

Macluraxanthone (**4**). Yellow crystal; M.P. 174–175 °C (lit. 170–172 °C [13]); Spectral data were consistent with published data [13].

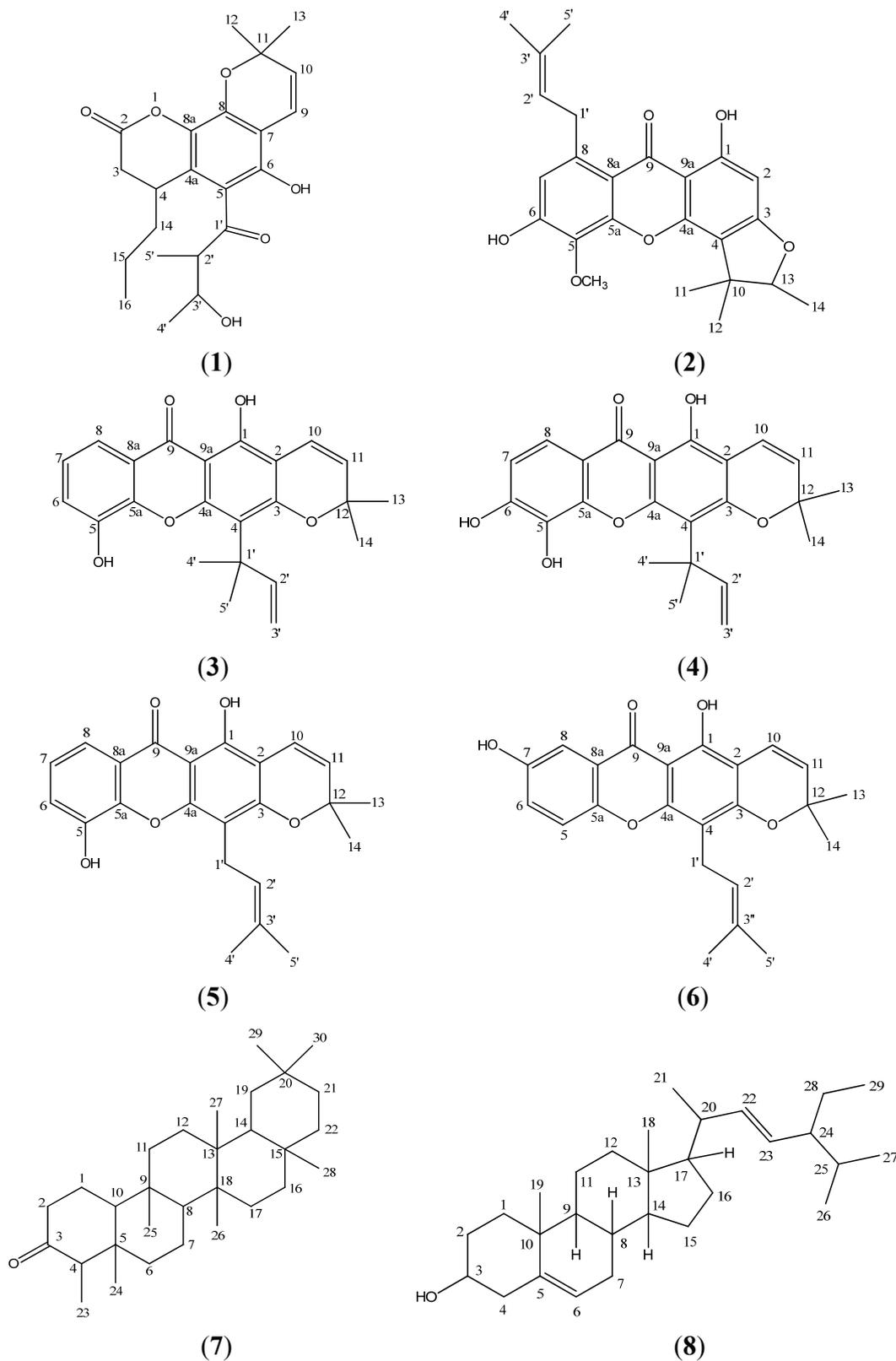
Trapezifolixanthone (**5**). Yellow crystal; M.P. 171–172 °C (lit. 171–172 °C [15]); Spectral data were consistent with published data [15].

Brasixanthone B (**6**). Yellow crystal; M.P. 227–229 °C (lit. 227–229 °C [16]); Spectral data were consistent with published data [16].

Friedelin (**7**). White needles; M.P. 245–246 °C (lit. 246–248 °C [2]); Spectral data were consistent with published data [2].

Stigmasterol (**8**). White needles; M.P. 155–157 °C (lit. 168–169 °C [17]); Spectral data were consistent with literature data [17].

Figure 3. Structures of soulamarin (**1**), caloxanthone B (**2**), caloxanthone C (**3**), macluraxanthone (**4**), trapezifolixanthone (**5**), brasixanthone B (**6**), friedelin (**7**) and stigmasterol (**8**).



4. Conclusions

The stem bark of *Calophyllum soulattri* furnished one new pyranocoumarin, soulamarin (**1**), together with five xanthenes caloxanthone B (**2**), caloxanthone C (**3**), macluraxanthone (**4**), trapezifolixanthone (**5**) and brasixanthone (**6**), a common triterpene, friedelin (**7**), and the steroidal triterpene stigmasterol (**8**).

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Sample Availability: Samples of all the compounds are available from the authors.

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