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NOTE

A new prenylated dihydrochalcone from the leaves of *Artocarpus lowii*

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Abstract A new prenylated dihydrochalcone, 2′,4′-dihydroxy-4-methoxy-3′-prenyldihydrochalcone (**1**), along with two known compounds, 2′,4′,4-trihydroxy-3′-prenylchalcone (**2**) and 2′,4-dihydroxy-3′,4′-(2,2-dimethylchromene)chalcone (**3**) were isolated from the leaves of *Artocarpus lowii*. The structures of **1–3** were elucidated by spectroscopic methods and by comparison with data reported in the literature. Compounds **1–3** showed strong free radical scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH) measured by electron spin resonance (ESR) spectrometry.

Keywords *Artocarpus lowii* · Moraceae · Leaves · Chalcone · Antioxidant · DPPH · ESR

Introduction

The genus *Artocarpus* (Moraceae) is represented by 20 species among the flora of Peninsular Malaysia, including cultivated species. Three species, i.e., *Artocarpus communis* (locally known as "sukun"), *A. heterophyllus* ("nangka") and *A. integer* ("chempedak") are cultivated throughout the country for their edible fruits [1, 2]. Phytochemical studies on *Artocarpus* plants have found various phenolic compounds, especially prenylated flavonoids [3, 4]. Some of the flavonoids have shown interesting biological properties, including cytotoxicity [5, 6], free radical scavenging [7] and antiinflammatory properties [8, 9]. Our previous studies on the bark of *A. scortechinii* King and *A. teysmanii* Miq. from Malaysia have resulted in the isolation of several flavonoids [10, 11]. During continuing research on Malaysian *Artocarpus* plants, we investigated the leaves of *A. lowii* King. This plant, locally known as "miku," is a rare species in Malaysia. The sap is traditionally used as an ointment and as cooking oil by old folk [1]. Although there are many reports on the phytochemicals and biological studies of the *Artocarpus* plants, this is believed to be the first study on *A. lowii* King. This paper reports the isolation and structural elucidation of the new compound **1** as well as the free radical scavenging activities of compounds **1–3** measured by ESR.

Results and discussion

Purification of the petroleum ether and dichloromethane extracts of the leaves of *Artocarpus lowii* have resulted in the isolation of one new prenylated dihydrochalcone, 2′,4′-dihydroxy-4-methoxy-3′-prenyldihydrochalcone (**1**) (Fig. 1). Further purification of the dichloromethane extract afforded two known compounds, 2′,4′,4-trihydroxy-3′-prenyl-chalcone (**2**) and 2′,4-dihydroxy-3′,4′-(2,2-dimethylchromene)chalcone (**3**).

Compound **1** was isolated as pale yellow powder, mp 91.1–92.3 °C and gave a dark gray spot with an ethanolic ferric chloride solution. The molecular formula was

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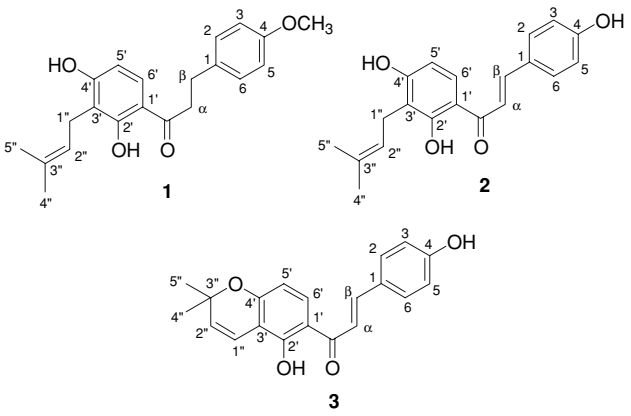

Fig. 1 Structures of compounds **1**, **2** and **3**

determined to be $C_{21}H_{24}O_4$ from its HRFABMS. The IR spectrum showed absorption bands for hydroxyl (3,419 cm^{-1}) and chelated carbonyl groups (1,618 cm^{-1}). The UV spectrum showed maximum absorptions at 335, 296 and 234 nm in MeOH, indicating a chalconoid structure. The lack of bathochromic shift upon the addition of NaOMe showed that C-4 at the B ring is substituted [12]. The ^{13}C NMR (Table 1) and DEPT spectra revealed the presence of 21 signals, including those for one carbonyl group (δ 205.2), three methylene carbons (δ 22.2, 30.2 and 40.2) and two methyl groups (δ 17.9 and 25.8), corresponding to a prenylated chalcone. The ^1H-NMR spectrum (Table 1) indicated signals for a chelated phenolic proton [δ 13.15 (1H, s)], a methoxyl group [δ 3.74 (3H, s)], a 3,3-dimethylallyl group [δ 5.24 (1H, t, $J = 7.3$ Hz), 3.33 (2H, d, $J = 7.3$ Hz), 1.62 (3H, s), and 1.75 (3H, s)], *ortho*-coupled aromatic protons [δ 6.47 (1H, d, $J = 8.9$ Hz) and 7.68 (1H, d, $J = 8.9$ Hz)] and A_2B_2 aromatic protons [δ 6.83 (2H, d, $J = 8.9$ Hz) and 7.20 (2H, d, $J = 8.9$ Hz)]. The presence of two triplets upon integrating for 2H with $J = 7.6$ Hz each at δ 2.95 and δ 3.25 and the absence of typical *trans*-olefinic protons of a chalcone in the downfield region suggest that compound **1** is a dihydrochalcone. On the basis of HMQC and HMBC spectral analysis, all protons and carbon signals were fully assigned, and the positions of the substituents on the aromatic rings were determined. The HMBC correlations for 2′-OH/C-1′, C-2′ and C-3′, 4′-OH/C-3′, C-4′ and C-5′ substantiated that the hydroxyl groups are located at C-2′ and C-4′. The HMBC correlations for H-1″/C-2′, C-3′, and C-4′ confirmed that the 3,3-dimethylallyl group is located at C-3′ in **1**. In turn, the HMBC correlations for methoxyl protons and C-4 established that the methoxyl group is located at C-4. These assignments confirmed the positions of the 3,3-dimethylallyl substituent, the hydroxyl groups and the methoxyl group in the structure of **1**. Thus, the structure of **1** was deduced as 2′,4′-dihydroxy-4-methoxy-3′-prenyldihydrochalcone.

Table 1 ^1H-NMR (500 MHz) and ^{13}C NMR (125 MHz) data for **1** in acetone-d_6 (J values in parentheses)

Position	**1**	
	^1H	^{13}C
1		134.0
2	7.20 (1H, d, $J = 8.9$ Hz)	130.2
3	6.83 (1H, d, $J = 8.9$ Hz)	114.6
4		159.1
5	6.83 (1H, d, $J = 8.9$ Hz)	114.6
6	7.20 (1H, d, $J = 8.9$ Hz)	130.2
α	3.25 (2H, t, $J = 7.6$ Hz)	40.2
β	2.95 (2H, t, $J = 7.6$ Hz)	30.2
C = O		205.2
1′		113.8
2′	13.15 (1H, s, OH)	163.8
3′		115.9
4′	9.24 (1H, s, OH)	162.5
5′	6.47 (1H, d, $J = 8.9$ Hz)	108.0
6′	7.68 (1H, d, $J = 8.9$ Hz)	130.6
1″	3.33 (2H, d, $J = 7.3$ Hz)	22.2
2″	5.24 (1H, t, $J = 7.3$ Hz)	123.2
3″		131.5
4″	1.62 (3H, s)	25.8
5″	1.75 (3H, s)	17.9
OMe	3.74 (3H, s)	55.4

Structure determination for compounds **2**–**3** was based on comparison with their spectroscopic data from literature values [13, 14]. These two known compounds were identified as 2′,4′,4-trihydroxy-3′-prenylchalcone or isobavachalcone (**2**) and 2′,4-dihydroxy-3′,4′-(2,2-dimethylchromene)chalcone or 4-hydroxylonchocarpin (**3**). However, to the best of our knowledge, this is the first report on the isolation of **2** and **3** from *Artocarpus* species. Compound **2** was previously isolated from *Anthyllis hermanniae* (Fabaceae) [15], *Glycyrrhiza glabra* (Leguminosae) [16], and *Dorstenia kameruniana* (Moraceae) [13], while compound **3** was previously found in *Glycyrrhiza glabra* [16], *Dorstenia mannii* [14], as well as *D. prorepens* and *D. zenkeri* [17].

Compounds **1**–**3** showed strong scavenging action against the DPPH radical. Treatment of the ethanolic solution of DPPH (25 mM) with 7.8 μg/ml solution of **1**, **2** and **3** was observed by an ESR. Vitamins C and E were used as positive controls. The DPPH radical was scavenged 61.9, 64.4, 41.6, 60.2 and 48.6% by Vitamins C, E, **1**, **2** and **3**. The IC_{50} values of **1**, **2** and **3** were determined to be 0.24, 0.03 and 0.15 mM, respectively. Compound **2** was found to be more active in the free radical scavenging reactions, presumably due to the presence of more hydroxyl groups compared to compounds **1** and **3**.

Experimental

General experimental procedures

Melting points were determined on a micro-melting point apparatus and were uncorrected. UV spectra were recorded on a Shimadzu (Tokyo, Japan) UV-1601PC spectrophotometer. IR spectra were measured on a Perkin Elmer (Wellesley, MA, USA) 1650 FTIR spectrometer. All NMR spectra (^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC) were recorded on a JEOL (Tokyo, Japan) JNM A500 NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). The ESR spectra were recorded on a JEOL JES-FA100 ESR spectrometer using manganese dioxide as an internal standard. VLC was carried out using Merck (Darmstadt, Germany) silica gel 60 (230–400 mesh), gravity column chromatography with Merck silica gel 60 (70–230 mesh) and Sigma (St. Louis, MO, USA) Sephadex LH-20.

Plant material

Leaves of *A. lowii* were collected in May 2003 from Gombak Rain Forest, Selangor, Malaysia. A voucher specimen (UKMB 03995) was deposited at the Herbarium of Universiti Kebangsaan Malaysia, Bangi, Selangor.

Extraction and isolation

Sequential extractions of the powdered leaves of *A. lowii* (2 kg) were carried out using 5 l each of petroleum ether and CH_2Cl_2 for 48 h. The extracts were filtered and concentrated to dryness, to afford petroleum ether crude extract (20 g) and CH_2Cl_2 crude extract (28 g). The petroleum ether crude extract of the leaves (18 g) was subjected to Si gel VLC (200 g) and eluted under gradient conditions with increasing amounts of EtOAc in hexane to give twelve fractions. Fractions with a similar pattern on TLC were combined to give four major fractions, A to D. Fraction C (2.5 g) was further purified by Si gel column chromatography (150 g) using hexane–EtOAc (7:3) as an eluent, followed by Sephadex LH-20 (20 g, MeOH) to yield **1** (117.5 mg). The CH_2Cl_2 crude extract of the leaves (20 g) was also subjected to Si gel VLC (220 g) and eluted by hexane, hexane–CH_2Cl_2, CH_2Cl_2–EtOAc, EtOAc in order of increasing polarity to afford 12 fractions. Fractions with a similar pattern on TLC were combined to give three major fractions, A_d–C_d. Fraction B_d (3.5 g) was subjected to Si gel column chromatography (150 g) using hexane–EtOAc (7:3) as an eluent, followed by Sephadex LH-20 (15 g, MeOH) to give more of **1** (25.2 mg). Fraction C_d (3.0 g) was purified by Si gel column chromatography (150 g) using hexane–EtOAc (7:3), followed by Sephadex LH-20 (15 g, MeOH) to afford **2** (1.8 g) and **3** (6.3 mg).

2′,4′-Dihydroxy-4-methoxy-3′-prenyldihydrochalcone (**1**): pale yellow powder; UV (MeOH) λ_{max} nm (log ε): 335 (4.09), 296 (4.15), 234 (4.26); UV (MeOH + NaOMe) λ_{max} (log ε) nm: 337 (4.47), 255 (3.93); IR (KBr) cm^{-1}: 3,419, 1,618, 1,589, 1,516, 1,294. HRFAB-MS *m/z*: 341.1727 (Calcd 341.1753 for $C_{21}H_{25}O_4$). ^{1}H and ^{13}C NMR data are given in Table 1.

DPPH electron spin resonance (ESR) assay

Each sample stock solution (1.0 mg/ml) was diluted to final concentrations of 500, 250, 125, 62.50, 31.25, 15.63 and 7.82 μg/ml, in absolute ethanol. A total of 200 μl of 25 mM DPPH ethanolic solution was added to 200 μl sample solution of different concentrations in eppendorf tubes. The reaction mixture was shaken for 10 s and then transferred to a flat cell and fitted into the cavity of the ESR spectrometer. The spin adduct was measured on an ESR spectrometer exactly 30 s later. The ethanolic DPPH solution was used as control, while vitamins C and E were used as positive controls. The conditions of the ESR spectrometer were set at temperature, 298 K; magnetic field, 336 mT; modulation frequency, 100 kHz; microwave frequency, 9.42 GHz; microwave power, 0.998 mW, and sweep time, 30 s.

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