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Naturally occurring prenylated coumarins from *Micromelum integerrimum* twigs

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ABSTRACT

A phytochemical investigation of secondary metabolites from *Micromelum integerrimum* was reported. Two new prenylated coumarins named as hydramicromelin D (**1**) and integerrimelin (**2**) were isolated along with 9 known coumarin derivatives. The chemical structures as well as relative stereochemistries of those coumarins were confirmed on the basis of extensive spectroscopic data interpretation and comparison with those previously published data.

1. Introduction

A number of naturally occurring coumarins and prenylated coumarins have been continuously isolated from plants, particularly from the Apiaceae and Rutaceae families. *Micromelum* genus is classified as one of the 148 genera in Rutaceae family and it comprises about 21 species. *Micromelum integerrimum*, wildly distributed in South and Southeast Asia, is one of the species in *Micromelum* genus. Previous phytochemical investigations of *M. integerrimum* and its genus revealed that the secondary metabolites are highly diversified. Apart from acridone and carbazole alkaloids, a variety of coumarin derivatives, especially 6,7-di and 7,8-disubstituted coumarin core structures having at least a prenyl unit have been isolated (Cassady et al., 1979; Das et al., 1984; He et al., 2001; Ito et al., 2000; Kamperdick et al., 1999; Lekphrom et al., 2011; Luo et al., 2009b; Rahmani et al., 1994). Interestingly, naturally occurring coumarin derivatives derived from *Micromelum* exhibited a variety of biological activities including anti-corpulence, cytotoxicity (Hirata et al., 2009), anti-platelet (Chen et al., 2003), and anti-mutagenicity (Nakahara et al., 2002). In term of searching for newly unique structure and on continuation of our interest in the chemistry of *Micromelum* genus, the chemical investigation of secondary metabolites from *M. integerrimum* was described. Two new coumarins with different oxidative cyclized isoprene unit together with 9 known coumarins were reported herein.

2. Results and discussion

Hydramicromelin D (**1**), integerrimelin (**2**) (Fig. 1) and nine known coumarin derivatives including hydramicromelin A (He et al., 2001), hydroxy-8-(2′,3′-dihydroxy-3′-methylbutyl)-coumarin (Ceccherelli et al., 1989), tortuoside (Ceccherelli et al., 1989), isoscopoletin (Shafizadeh and Melnikoff, 1970), integerriminol (Phakhodee et al., 2013), micromelin (Cassady et al., 1979), murragatin (Talapatra et al., 1973), minumicromelin acetonide (Ito et al., 1990), and murralogin (Talapatra et al., 1973) were isolated from *M. integerrimum*. The structural determination of these compounds was derived by intensive interpretation of spectroscopic data.

Compound **1**, $[\alpha]_D^{26}$ +59.90 (*c* 0.10, $(CH_3)_2CO$), was isolated as white solid and determined to have the molecular formula $C_{15}H_{14}O_7$ based on the TOF-HRMS data (*m*/*z* 329.0639, calcd for $C_{15}H_{14}O_7Na$ 329.0632). The IR absorption peaks suggested the presence of hydroxyl group as well as two kinds of ester functionalities at 3418, 1783, and 1729 cm^{-1}, respectively. The core structure of this molecule was determined by intensive analysis of NMR spectra. The ^1H NMR signals of a typical AB system at δ_H 7.95 (d, *J* = 9.6 Hz, 1H), 6.27 (d, *J* = 9.6 Hz, 1H) and AX system at δ_H 7.69 (s, 1H), 7.04 (s, 1H) indicated a characteristic of 6,7-disubstituted coumarin nucleus (He et al., 2001; Luo et al., 2009a; Phakhodee et al., 2013). The ^{13}C NMR resonances at δ_C 122.4 and 160.0 revealed that one carbon of this coumarin core structure was

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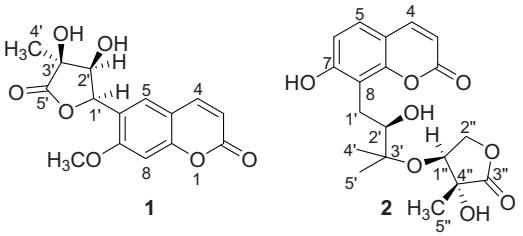

Fig. 1. Phytochemical structures of secondary metabolites isolated from twigs of *M. integerrimum*.

connected with carbon and another one was attached to an oxygen atom. The ^{1}H NMR and ^{13}C NMR of an oxidized isoprenyl unit resonated at δ_H 5.52 (d, J = 5.6 Hz, H-1′)/δ_C 79.5, 5.01 (s, OH), 4.94 (s, OH), 4.26 (d, J = 5.6 Hz, H-2′)/δ_C 77.5 and 1.38 (s, CH_3)/δ_C 21.1 as shown in Table 1. This unit was linked to C-6 (δ_C 122.4) of the coumarin framework as indicated from ^{2}J to ^{3}J heteronuclear multiple bond connectivity (HMBC) correlations between H-1′ (5.52) and the carbons at C-5 (δ_C 127.2), C-6 (δ_C 122.4), C-7 (δ_C 160.0) C-3′ (δ_C 72.4) and C-5′ (δ_C 175.6), and between H-2′ and C-6 (δ_C 122.4), C-1′ (δ_C 79.5) and C-5′ (δ_C 175.6) as depicted in Fig. 2. The ^{3}J HMBC coupling of OCH_3 (δ_H 4.02) with C-7 (δ_C 160.0) indicated that the methoxy group was attached to C-7 of the coumarin framework. From the ^{1}H and ^{13}C NMR spectral data described in Table 1, structure of compound **1** was deduced as an oxidative cyclized prenylated coumarin derivative similar to those of hydramicromelins A, B, and C (He et al., 2001). Nevertheless, the spin–spin coupling between H-1′and H-2′ (d, J = 5.6 Hz for compound **1**; d, J = 6.6 Hz for hydramicromelin A; d, J = 7.2 Hz for hydramicromelin B and dd, J = 2.9, 0.7 Hz and br s for hydramicromelin C) as well as the ^{13}C chemical shifts at C-2′, C-3′, C-4′ and C-5′ of **1** and those for hydramicromelins A, B and C are slightly different. The ^{13}C NMR data of **1** displayed signals of C-2′, C-3′, C-4′ and C-5′ at δ_C 77.5, 72.4, 21.1 and 175.6, respectively, while those corresponding carbons of hydramicromelins A, B, and C resonated at δ_C 79.4, 73.6, 22.3 and 177.6, δ_C 80.6, 76.5, 19.1 and 17.1, and δ_C 76.9, 77.6, 19.2 and 178.5 (He et al., 2001). This result implied that compound **1** is one of the oxidative cyclized prenylated coumarin isomers. The relative stereochemistries at C-1′, C-2′ and C-3′ of **1** were further determined by NOESY experiment in which the correlations of H-2′ with H-1′ and H-4′ indicated that these protons were located in the same orientation as illustrated in Fig. 2. Thus, compound **1** was named as hydramicromelin D.

Compound **2** was obtained as light yellow gum with positive optical rotation ($[\alpha]_D^{26}$: +116.50, in acetone). It gave a quasimolecular ion peak [M+Na$^+$] at m/z 401.1217 (calcd for $C_{19}H_{22}O_8Na$ 401.1212) corresponding to the molecular formula $C_{19}H_{22}O_8$, with 9 degrees of unsaturation. The IR spectra displayed absorption peaks at 3385, 1763, and 1703 cm^{-1} corresponding to hydroxyl, α,β-unsaturated lactone, and γ-lactone functionalities. ^{1}H NMR of **2** showed AB system of α,β-unsaturated lactone moiety at δ_H 7.86 (d, J = 9.6 Hz, H-4), 6.16 (d, J = 9.6 Hz, H-3) similar to those of hydramicromelin D (**1**). The difference is that compound **2** exhibited AB system of aromatic ring at δ_H 7.39 (d, J = 8.8 Hz, H-5), 6.86 (d, J = 8.8 Hz, H-6) instead of AX system observed in compound **1**. The ^{1}H NMR and ^{13}C NMR revealed a typical isoprenylated unit at δ_H 3.70 (dd, J = 10.0, 1.6 Hz, H-2′)/δ_C 79.4 (C-2′), 3.33 (dd, J = 14.0, 1.6 Hz, H-1a′) and 2.80 (dd, J = 14.0, 1.0 Hz, H-1b′)/δ_C 25.3 (C-1′), 1.31 (s, H-4′)/δ_C 25.0 (C-4′), 1.29 (s, H-5′)/δ_C 24.2 (C-5′). The γ-lactone moiety [(δ_H 4.43 (dd, J = 10.6, 4.4 Hz, H-2a″) and 4.12 (d, J = 10.6, H-2b″)/δ_C 71.3 (C-2″), 4.12 (d, J = 10.6, H-1″)/δ_C 73.2 (C-1″) and 1.38 (s, CH_3) was also observed. The linkage between the coumarin framework and the isoprenylated unit was confirmed based on intensive analysis of HMBC spectral data. ^{2}J and ^{3}J HMBC correlations between H-1a′ (δ_H 3.33) and C-7 (δ_C 160.4), C-8 (δ_C 114.5), C-8a (δ_C 153.8) and between H-1b′ (δ_H 2.80) and C-7 (δ_C 160.4), C-8 (δ_C 114.5), C-8a (δ_C 153.8), C-2′ (δ_C 79.4), C-3′ (δ_C 71.9) indicated that the isoprenylated unit was located at C-8 of coumarin nucleus. The attachment of γ-lactone unit at C-3′ of the isoprenylated moeity was also verified by HMBC spectrum in which correlation was observed between proton signal of H-1″ (δ_H 4.12) and carbons at C-3′ (δ_C 71.9), C-3″ (δ_C 177.4), C-4″ (δ_C 72.1) and C-5″ (δ_C 20.7). The relative configurations of **2** were deduced by ^{1}H NMR and NOESY experiments. The proton signal of H-1b′ at δ_H 2.80 bearing large coupling constants of 14.0 and 10.0 Hz was assigned as geminol coupling to H-1a′ and *cis*-vicinol coupling to H-2′, respectively. This information was further supported by the key NOESY correlations as illustrated in Fig. 2. Moreover, the relative configurations of the remaining stereogenic centres on

Table 1
^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (acetone-d_6).

Position	**1**		**2**	
	δ_C, mult	δ_H (J in Hz)	δ_C, mult	δ_H (J in Hz)
2	160.7, C	–	160.3, C	–
3	113.2, CH	6.27, d (9.6)	111.4, CH	6.16, d (9.6)
4	143.7, CH	7.95, d (9.6)	144.4, CH	7.86, d (9.6)
4a	112.1, C	–	111.9, C	–
5	127.2, CH	7.69, s	127.1, CH	7.39, d (8.8)
6	122.4, C	7.04, s	113.4, CH	6.86, d (8.8)
7	160.0, C	–	160.4, C	–
8	99.5, CH	–	114.5, C	–
8a	156.2, C	–	153.8, C	–
1′	79.5, CH	5.52, d (5.6)	25.3, CH_2	2.80, dd (14.0, 10.0) 3.33, dd (10.0, 1.6)
2′	77.5, CH	4.26, d (5.6)	79.4, CH	3.70, dd (10.0, 1.6)
3′	72.4, C	–	71.9, C	–
4′	21.1, CH_3	1.38, s	25.0, CH_3	1.31, s
5′	175.6, C	–	24.2, CH_3	1.29, s
1″	–	–	73.2, CH	4.12, d (10.6)
2″	–	–	71.3, CH_2	4.12, d (10.6) 4.43, dd (10.6, 4.4)
3″	–	–	177.4, C	–
4″	–	–	72.1, C	–
5″	–	–	20.7, CH_3	1.38, s
OCH_3	56.1, CH_3	4.02, s	–	–
OH	–	5.01	–	–
OH	–	4.94	–	–