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Three New Coumarins from *Micromelum integerrimum*

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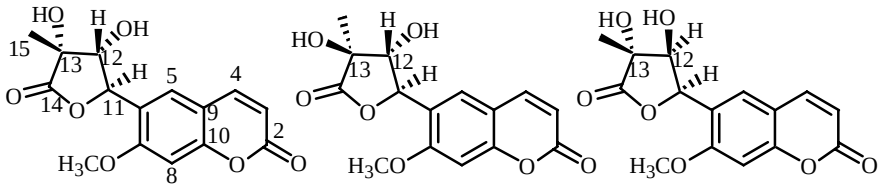
Abstract: Three derivatives of micromelin, named hydramicromelins A-C (**1-3**), were isolated from the aerial part of *Micromelum integerrimum* (Buch.-Ham) Roem., respectively. Hydramicromelins A-C (**1-3**) were epimers which possessed the same plane structure and molecular formula. Their structures were elucidated based on MS and NMR data. The relative configurations of **1-3** were established by NOE analysis.

Keywords: *Micromelum integerrimum*, Rutaceae, coumarin, micromelin, hydramicromelins A-C.

Micromelum integerrimum (Rutaceae) is a shrub growing in Yunnan Province. Leaves and barks of this plant have been used for the treatment of dysentery, and arthritis[1]. Previous researches revealed that this plant contained coumarins[2-8]. Micromelin, the main chemical constituent of this plant, showed significant inhibition against leukaemia P-388 cell line (T/C 149% at 10 mg/kg) but was inactive in lower doses, and also showed significant activity (T/C 228% at 1.25 mg/kg) against Lewis lung carcinoma[3]. In order to search for more active components from this plant, the chemical constituents of the plant was investigated. This paper described the isolation and structure elucidation of three new coumarins, derivatives of micromelin, named hydramicromelins A-C (**1-3**) from the ethanolic extract of the aerial part of *M. integerrimum* collected in Xishuangbanna, Yunnan Province. Their structures were elucidated on the basis of 1D and 2D NMR

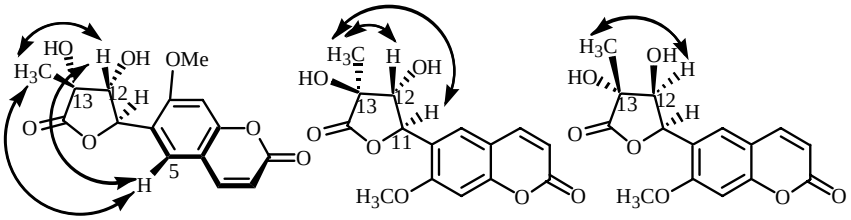
Figure 1 The relative configurations of hydramicromelins A-C (1-3)



experiments (HMQC, HMBC, ^{1}H-^{1}H COSY). The relative configurations of **1**-**3** were established by analysis of the NOE experiment.

Compound **1**, $[\alpha]_D^{26}$ +125.0 (*c* 4.6, C_5H_5N), was determined to have the molecular formula $C_{15}H_{14}O_7$ based on HREIMS (*m*/*z* 306.0730 $[M]^+$, calcd: 306.0740). The UV spectrum (222, 246, 291.5, 322.5 nm) and IR (KBr) data (1732, 1620, 1564 cm^{-1}) suggested the presence of coumarin nucleus, which was further supported by characteristic signals of a typical AB system at δ_H 6.36, 7.69 (d, 1H each, 9.5) assigned to H-3, H-4 (**Table 1**)[9,10]. The ^{13}C NMR spectra (**Table 1**) revealed the presence of a 5-carbon moiety and a methoxyl group (δ_C 56.3q) besides the coumarin chromophore, suggesting **1** was an analogue of micromelin[2-5]. The proton and carbon resonances at δ_H 7.78 (s) and 6.89 (s), and δ_C 127.7 (d) and 99.9 (d) indicated that **1** had an oxygen-substitution at C-7 and carbon-substitution at C-6. In the HMBC experiment, the ^{1}H-^{13}C long-range correlations between δ_H 1.78 (corresponding carbon at δ_C 22.3q) and δ_C 73.6s, 79.4d, 177.6s, and between δ_H 4.67 (δ_C 79.4d) and δ_C 73.6s, 79.9d, 123.2s, 177.6s, and δ_H 6.15 (δ_C 79.9d) and δ_C 123.2s, 127.7d, 161.2s, proved that a 5-carbon moiety was linked with C-6 *via* C-11. The ^{1}H-^{13}C long-range correlations between δ_H 6.15 and δ_C 177.6s revealed that the 5-carbon moiety was a cyclized side chain which was similar to that in micromelin. The ^{13}C NMR signals at δ 79.9d, and 79.4s revealed that C-12 and C-13 were hydroxyl-substituted, rather than an epoxy between C-12 and C-13, and supported by its IR data (3422 cm^{-1}) as well. Moreover, in the HMBC experiment, the ^{1}H-^{13}C long-range correlations between δ_H 3.67 (OMe) and δ_C 161.2 (C-7) indicated that the methoxyl was linked at C-7. A strong NOE was observed between Me-13 and H-5, indicating Me-13 was in β-form (**Figure 2**), at the same time, the NOE effect between H-12 and Me-13 and H-5 revealed that H-12 was also in β-form, therefore, the hydroxyls at C-12 and C-13 were both in α-form. Thus, **1** was identified as 12 α, 13α –dihydroxymicromelin as shown in **Figure 1**, and named hydramicromelin A (**1**).

Figure 2 The key NOE of hydramicromelins A-C (**1**–**3**)



1 **2** **3**

2, $[\alpha]_D^{27}$ +10.46 (*c* 8.0, C_5H_5N), was determined to have the molecular formula $C_{15}H_{14}O_7$ based on HREIMS (*m*/*z* 306.0735 $[M]^+$, calcd: 306.0740). The ^{13}C NMR and DEPT spectra (**Table 1**) of **2** showed fifteen carbon signals including two methyl (one OMe and C-Me), six methine, and seven quaternary carbon atoms. The UV (221, 247, 291, 328 nm), IR data (1731, 1626, 1565 cm^{-1}) and ^{1}H NMR [δ_H 6.32, 7.57 (1H each, d, 9.5)] suggested the presence of a coumarin nucleus[9,10]. The ^{13}C NMR signals at δ_C 80.6d and 76.5s revealed a hydroxyl at C-12, and C-13, similar to **1**. The presence of a

cyclized side chain and substitution pattern as **1** was supported by its NMR spectra, HMQC and HMBC. Thus, **2** and **1** possessed the same plane structure. The coupling between H-11 (d, 7.2) and H-12 (d, 7.2), similar to those of **1** revealed that H-12 was in β-form and OH (C-12) was in α-form. The difference between **2** and **1** was the ^{13}C NMR signals for C-13, C-14 and C-15 of the 5-carbon cyclized side chain (**Table 1**), which suggested that **2** was a C-13 epimer of **1**. This was supported by the NOE effect between H-15 and H-11 (**Figure 2**). Therefore, **2** was determined to be 12α, 13β-dihydroxymicromelin, and named hydramicromelin B (**Figure 1**).

Table 1. The ^{1}H and ^{13}C NMR assignments for hydramicromelins A-C (**1-3**) in C_5D_5N

No.	**1** [a]		**2** [a]		**3** [a]	
	^{1}H [b]	^{13}C	^{1}H [b]	^{13}C	^{1}H [b]	^{13}C
2	/	160.7s	/	160.6s	/	161.0
3	6.36 (d, 9.5)	113.8d	6.32 (d, 9.5)	113.7d	6.34 (d, 9.5)	113.7
4	7.69 (d, 9.5)	143.8d	7.57 (d, 9.5)	143.7d	7.65 (d, 9.5)	144.3
5	7.78 (s)	127.7d	7.66 (s)	128.4d	7.86 (d, 0.7)	128.0
6	/	123.2s	/	123.4s	/	122.1
7	/	161.2s	/	161.2s	/	159.7
8	6.89 (s)	99.9d	6.85 (s)	99.9d	6.69 (s)	99.2
9	/	112.7s	/	112.6s	/	112.6
10	/	156.5s	/	156.5s	/	156.1
11	6.15 (d, 6.6)	79.9d	5.84 (d, 7.2)	79.1d	6.60 (dd, 2.9, 0.7)	80.0
12	4.67 (d, 6.6)	79.4d	5.13 (d, 7.2)	80.6d	4.94 (br s)	76.9
13	/	73.6s	/	76.5s	/	77.6
14	/	177.6s	/	179.1s	/	178.5
15	1.78 (s, 3H)	22.3q	1.94 (s, 3H)	19.1q	2.03 (s, 3H)	19.2
7-OMe	3.67 (s, 3H)	56.3q	3.65 (s, 3H)	56.3q	3.63 (s, 3H)	56.3

[a] ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, and assigned by the ^{1}H-^{1}H COSY, HMQC and HMBC experiments.
[b] Coupling constants were presented in Hertz, unless otherwise indicated, all proton signals integrate to 1H.

The HREIMS (*m/z* 306.0730 $[M]^+$, calcd: 306.0740) of **3**, $[\alpha]_D^{18}$ -17.02 (*c* 3.8, C_5H_5N) gave the molecular formula $C_{15}H_{14}O_7$. The IR data (1730, 1627, 1565 cm^{-1}), and the ^{1}H NMR signals at δ 6.34 and 7.65 (d, 1H each, 9.5) assigned to H-3 and H-4 (**Table 1**) revealed a coumarin lactone similar to those of **1** and **2**. The ^{13}C NMR and DEPT spectra (**Table 1**) of **3** showed fifteen carbon signals including two methyl (one OMe and one C-Me), six methine, and seven quaternary carbon atoms, which were similar to those of **1** and **2**. From the HMBC experiments, **3** had the same plane structure as **1** and **2**. The ^{1}H and ^{13}C NMR differences between **3** and **1** were observed for C-11, C-12, C-13, and C-15, especially for the coupling between H-11 at δ_H 6.60 (dd, 0.7 and 2.9) and H-12 at δ_H 4.94 (brs) (**Table 1**), suggesting that H-11 and H-12 of **3** were in *cis*-form. The NOE between H-12 and Me-13 similar to that of **2,** and the absence of NOE effect between H-11 and Me-13 similar to that of **1**, suggested that Me-13 was in β-form (**Figure 2**). The NOE between H-12 and Me-13 of **1-3** showed the presence of NOE between H-12 and Me-13 either their in *cis*-form or *trans*-form. Thus, **3** was characterized as 12β, 13α-dihydroxymicromelin, and named hydramicromelin C (**Figure 1**).

Acknowledgments

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