

## Chemical Constituents of *Micromelum minutum*. Isolation and Structural Elucidation of New Coumarins<sup>1)</sup>

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**The chemical constituents of an acetone extract of the stems of *Micromelum minutum* WIGHT et ARN (Rutaceae), collected at Nakorn-Rachasima province in Thailand, were studied. Six new coumarins, named micromarin-A (1), -B (2), -C (3), -F (4), -G (5), and -H (6), were isolated along with six known coumarins, and their structures were elucidated by chemical and spectroscopic methods.**

**Key words** coumarin; micromarin; *Micromelum minutum*; Rutaceae; isovalerate

*Micromelum minutum* (Rutaceae) is used for the treatment of fever and giddiness and as a poultice for ringworm and ague in Malaysia.<sup>2)</sup> Also it is used in the traditional folk medicine of Fiji.<sup>2)</sup> Some coumarins,<sup>3–9)</sup> a flavanone,<sup>9)</sup> a quinolone alkaloid,<sup>5)</sup> and a carbazole alkaloid,<sup>9)</sup> have been reported as constituents of plants of the genus *Micromelum*. In our search for anti-tumor promoters from medicinal plants, an acetone extract of the stems of *M. minutum* WIGHT et ARN (Rutaceae), collected in Nakorn-Rachasima province in Thailand, was found to exhibit anti-tumor-promoting activity. This paper describes the isolation and structural elucidation of six new coumarins named micromarin-A (1), -B (2), -C (3), -F (4), -G (5), and -H (6) from the stems of *M. minutum*.

An acetone extract of stems of the plant was chromatographed on silica-gel, eluting with hexane-acetone, followed by repeated preparative TLC to afford six new coumarins along with six known ones.

**Structure of Micromarin-A (1)** Micromarin-A (1), [ $\alpha$ ]<sub>D</sub> +3.44° (CHCl<sub>3</sub>), was obtained as a colorless powder. The molecular formula C<sub>20</sub>H<sub>22</sub>O<sub>6</sub> was established by analysis of high resolution (HR)-MS. The UV spectrum [ $\lambda_{\max}$  (log  $\epsilon$ ): 209 (4.82), 256 (4.14), 267 (4.03), 321 (4.60) nm], IR band [ $\nu_{\max}$  1732, 1606 cm<sup>-1</sup>], and <sup>1</sup>H-NMR spectra [ $\delta$  3.96 (3H, s, OCH<sub>3</sub>), 7.62 (1H, d,  $J$ =9.5 Hz), 6.26 (1H, d,  $J$ =9.5 Hz), 7.43 (1H, d,  $J$ =8.4 Hz), and 6.87 (1H, d,  $J$ =8.4 Hz)], coupled with a nuclear Overhauser effect (NOE) enhancement between the 7-OCH<sub>3</sub> ( $\delta$  3.96) and H-6 ( $\delta$  6.87), indicated the presence of a 7-methoxy-8-substituted coumarin nucleus in this molecule.<sup>10)</sup> The existence of an isovaleryloxy group in the molecule was confirmed by EI-MS [ $m/z$  257 (M<sup>+</sup>·OCOCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>)<sup>+</sup>] and <sup>1</sup>H-NMR spectrum [ $\delta$  2.25 (2H, d,  $J$ =7.3 Hz), 2.13 (1H, m), 0.95 (6H, d,  $J$ =6.7 Hz)]. Furthermore, in the <sup>1</sup>H-NMR spectrum, AB-type signals at  $\delta$  4.78 and 4.72 (each 1H, d) having a geminal coupling constant ( $J$ =13.2 Hz), two methine proton signals at  $\delta$  4.03 and 4.05 (each 1H, d,  $J$ =2.2 Hz), and two 1H-singlets at  $\delta$  5.54 and 5.37 assignable to methylene protons attached to an ester moiety, vicinal methines of an epoxide, and an *exo*-methylene group, respectively, were observed, indicating the

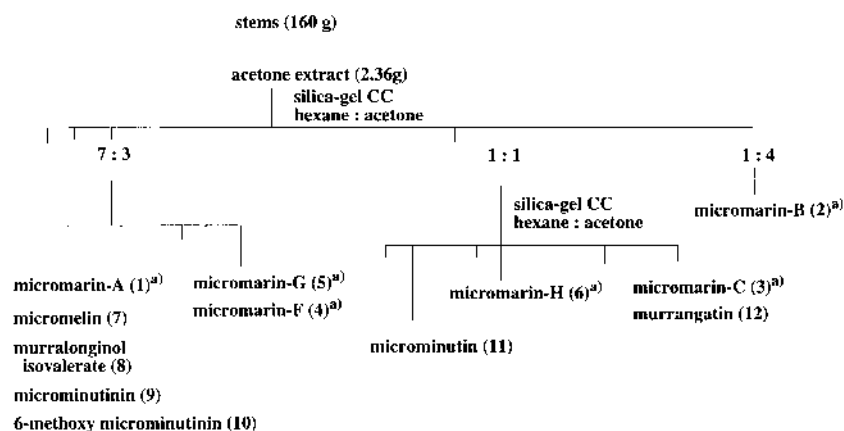
presence of the partial structure  $\begin{array}{c} \text{O} \\ | \\ \text{---C---C---C(=CH}_2\text{)--CH}_2\text{---O---} \\ | \quad | \\ \text{H} \quad \text{H} \end{array}$ .

*trans*-Orientation of the epoxide ring was proposed from the value of the coupling constant ( $J$ =2.2 Hz) of two vicinal protons, the same as that in the case of phebalosin.<sup>11,12)</sup>

Finally, connectivities of these structural units were established from the results of analyses of the <sup>1</sup>H-detected heteronuclear multiple bond connectivity (HMBC) spectrum shown by arrows in Fig. 1. The significant C–H long-range correlations for structure determination are described below. A carbon signal at  $\delta_C$  161.81 (C-7) showed three-bond correlations with proton signals at  $\delta_H$  7.43 (H-5), 3.96 (7-OCH<sub>3</sub>), and 4.03 (H-1'), indicating the presence of a side-chain at C-8 on the coumarin nucleus. One of the carbonyl carbons at  $\delta_C$  172.69 (C-1'') showed three-bond correlations with the methylene protons at  $\delta_H$  4.72 and 4.78 (H-5') on the side-chain which correlated with the methine carbon at  $\delta_C$  58.07 (C-2') on the epoxide. The *exo*-methylene carbon at  $\delta_C$  115.84 (C-4') correlated with the methine proton at  $\delta_H$  4.05 (H-2') and the methylene protons at  $\delta_H$  4.72 and 4.78 (H-5'). On the basis of the foregoing spectral data, structure **1** was assigned for micromarin-A.

**Structures of Micromarin-B (2) and -C (3)** These new coumarins were isolated as pale yellow oils, [ $\alpha$ ]<sub>D</sub> -1.64° (CHCl<sub>3</sub>) and [ $\alpha$ ]<sub>D</sub> -8.11° (CHCl<sub>3</sub>), respectively, and had the same molecular formula C<sub>20</sub>H<sub>24</sub>O<sub>7</sub> based on the HR-FAB-MS spectra of both compounds. The UV and IR spectra of these compounds closely resemble each other. In the <sup>1</sup>H-NMR spectra, both compounds showed characteristic signals assignable to a 7-methoxy-8-substituted coumarin nucleus and an isovaleryloxy group (Table 1). Moreover, the presence of an *exo*-methylene group [ $\delta$  5.02 (2H, br s);  $\delta$  5.34 (1H, br s), 5.33 (1H, br s)], two adjacent methines [ $\delta$  5.32, 4.65 (each 1H, d,  $J$ =8.4 Hz);  $\delta$  5.43 (1H, dd,  $J$ =8.1, 11.0 Hz), 4.64 (1H, dd,  $J$ =8.1, 6.6 Hz)] attached to a hydroxyl group [ $\delta$  3.79, 3.32 (each 1H, br);  $\delta$  3.68 (1H, d,  $J$ =11.0 Hz), 2.41 (1H, d,  $J$ =6.6 Hz)], and an allylic methylene [ $\delta$  4.57 (2H, br s);  $\delta$  4.77 (1H, d,  $J$ =13.2 Hz), 4.80 (1H, d,  $J$ =13.2 Hz)] bearing an ester group were suggested in each of the coumarins. These spectral data clearly show that both coumarins have the partial structure [–CH(OH)–CH(OH)–C(=CH<sub>2</sub>)–CH<sub>2</sub>–O–]. These spectral data and analyses of HMBC spectrum (see Experimental) suggested that these coumarins were diastereoisomeric isomers of each other involving their two oxygenated carbons. In order to confirm the structure of these coumarins, treatment of micromarin-A (1) with 1 N H<sub>2</sub>SO<sub>4</sub> in dioxane at room temperature gave two glycols **2'** and **3'**. One (**2'**) of them was found to be identical with natural **2** and the other with natural **3** by IR, <sup>1</sup>H-NMR, and co-TLC comparisons. Furthermore, treatment of these glycols

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Silica-gel CC and Preparative TLC were carried out with appropriate combinations of hexane, benzene, iso-Pr<sub>2</sub>O, Et<sub>2</sub>O, EtOAc, acetone, CHCl<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, and MeOH. a) New compounds.

Chart 1

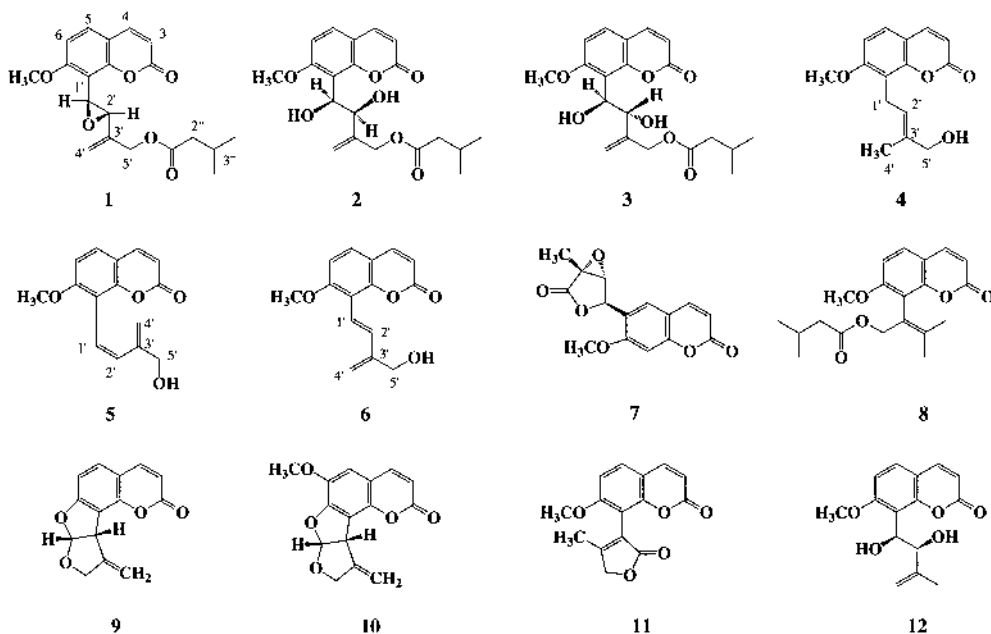


Chart 2

with acetone in the presence of a catalytic amount of toluene-*p*-sulphonic acid (*p*-TsOH) gave the corresponding acetonides, (**2a**) and (**3a**), respectively. For the reciprocal differential NOEs between H-1' ( $\delta$  6.13) and H-2' ( $\delta$  5.13) in micromarin-C acetonide (**3a**), 13 and 11% enhancements were observed. On the other hand, in the case of micromarin-B acetonide (**2a**), no NOE enhancement was observed, neither by irradiation of H-1' at  $\delta$  5.57 nor of H-2' at  $\delta$  5.21. Since this spectral evidence shows clearly that the vicinal protons adjacent to the oxygen atoms of the acetonide ring in the micromarin-C acetonide (**3a**) molecule should be *cis*, the structure of micromarin-C must be in the *erythro* form (**3**). Thus, micromarin-B should be in the *threo* form (**2**). The absolute stereochemistry of these coumarins remains undetermined.

**Structure of Micromarin-F (4)** Micromarin-F (**4**) was obtained as a colorless oil, C<sub>15</sub>H<sub>16</sub>O<sub>4</sub>. The 7-methoxy-8-sub-

stituted coumarin nucleus in this compound was also confirmed by the typical UV bands (see Experimental), and two pairs of AB-type doublets [ $\delta$  7.62 (H-4), 6.24 (H-3) (each 1H, d,  $J=9.5$  Hz) and  $\delta$  7.31 (H-5), 6.84 (H-6) (each 1H, d,  $J=8.4$  Hz)] and a 3H-singlet at  $\delta$  3.92 (7-OCH<sub>3</sub>) in the <sup>1</sup>H-NMR spectrum.<sup>10</sup> The remaining <sup>1</sup>H-NMR signals at  $\delta$  3.60 (2H, d,  $J=7.0$  Hz), 5.50 (1H, m), 1.89 (3H, s), and 3.99 (2H, s) and mass fragment peaks at  $m/z$  229 (M-·CH<sub>2</sub>-OH)<sup>+</sup> and 175 (M-·CH<sub>2</sub>-CH=C(CH<sub>3</sub>)-CH<sub>2</sub>-OH)<sup>+</sup> suggested the presence of [-CH<sub>2</sub>-CH=C(CH<sub>3</sub>)-CH<sub>2</sub>-OH] in the side-chain. Based on these spectral data, coupled with the appearance of NOE enhancement between the benzyl proton signal (H-1',  $\delta$  3.60) and a vinyl methyl proton signal (H-4',  $\delta$  1.89), and the results of the HMBC experiment (see Experimental), the structure of micromarin-F was concluded to be **4**.

Table 1. <sup>1</sup>H-NMR Data for the New Coumarins in CDCl<sub>3</sub>

	1	2	3	4	5	6
H-3	6.26 (d, 9.5)	6.25 (d, 9.5)	6.27 (d, 9.5)	6.24 (d, 9.5)	6.24 (d, 9.5)	6.28 (d, 9.5)
H-4	7.62 (d, 9.5)	7.62 (d, 9.5)	7.64 (d, 9.5)	7.62 (d, 9.5)	7.65 (d, 9.5)	7.63 (d, 9.5)
H-5	7.43 (d, 8.4)	7.41 (d, 8.8)	7.42 (d, 8.8)	7.31 (d, 8.4)	7.37 (d, 8.4)	7.32 (d, 8.4)
H-6	6.87 (d, 8.4)	6.88 (d, 8.8)	6.90 (d, 8.8)	6.84 (d, 8.4)	6.88 (d, 8.4)	6.87 (d, 8.4)
7-OCH <sub>3</sub>	3.96 (3H)	3.95 (3H)	3.97 (3H)	3.92 (3H)	3.95 (3H)	3.97 (3H)
H-1'	4.03 (d, 2.2)	5.32 (br d, 8.4)	5.43 (dd, 11.0, 8.1)	3.60 (2H, d, 7.0)	6.42 (d, 12.2)	7.44 (d, 17.1)
H-2'	4.05 (d, 2.2)	4.65 (d, 8.4)	4.64 (dd, 6.6, 8.1)	5.50 (m)	6.41 (d, 12.2)	7.00 (d, 17.1)
H-4'	5.54 (br)	5.02 (2H, br)	5.34 (br)	1.89 (3H)	4.87 (d, 1.7)	5.41 (d, 1.5)
	5.37 (br)		5.33 (br)		4.72 (d, 1.7)	5.36 (d, 1.5)
H-5'	4.78 (d, 13.2)	4.57 (2H, br)	4.80 (d, 13.2)	3.99 (2H)	4.22 (2H)	4.53 (2H)
	4.72 (d, 13.2)		4.77 (d, 13.2)			
Others	2.25 (2H, d, 7.3, H-2'')	2.19 (2H, d, 7.3, H-2'')	2.28 (2H, d, 7.3, H-2'')			
	2.13 (m, H-3'')	2.07 (m, H-3'')	2.14 (m, H-3'')			
	0.95	0.93	0.98			
	(6H, d, 6.7, 3''-CH <sub>3</sub> )	(6H, d, 6.7, 3''-CH <sub>3</sub> )	(6H, d, 6.7, 3''-CH <sub>3</sub> )			
		3.79 (br, 11-OH)	3.68 (d, 11.0, 11-OH)			
		3.32 (br, 12-OH)	2.41 (d, 6.6, 12-OH)			

Values in (δ) ppm. The coupling constants (*J*) in parentheses are in Hz. All signals correspond to 1H, and were observed as a singlet, unless otherwise stated.

Table 2. <sup>13</sup>C-NMR Data for the New Coumarins in CDCl<sub>3</sub>

	1	2	3	4
C-2	160.14 (s)	159.90 (s)	160.01 (s)	161.28 (s)
C-3	113.50 (d)	113.45 (d)	113.54 (d)	113.03 (d)
C-4	143.32 (d)	143.61 (d)	143.65 (d)	145.96 (d)
C-4a	112.85 (s)	113.13 (s)	113.25 (s)	112.98 (s)
C-5	129.10 (d)	128.78 (d)	128.60 (d)	127.23 (d)
C-6	107.55 (d)	107.78 (d)	107.87 (d)	107.31 (d)
C-7	161.81 (s)	160.09 (s)	160.37 (s)	160.22 (s)
7-OCH <sub>3</sub>	56.31 (q)	56.28 (q)	56.35 (q)	56.05 (q)
C-8	112.02 (s)	115.41 (s)	116.11 (s)	117.12 (s)
C-8a	153.97 (s)	152.88 (s)	153.25 (s)	152.87 (s)
C-1'	53.01 (d)	70.24 (d)	69.15 (d)	21.46 (d)
C-2'	58.07 (d)	75.90 (d)	75.89 (d)	122.50 (d)
C-3'	140.27 (s)	142.60 (s)	144.08 (s)	135.75 (s)
C-4'	115.84 (t)	115.17 (t)	116.59 (t)	13.83 (q)
C-5'	63.19 (t)	63.39 (t)	64.34 (t)	68.75 (t)
C-1''	172.69 (s)	172.68 (s)	172.97 (s)	
C-2''	43.34 (t)	43.26 (t)	43.33 (t)	
C-3''	25.65 (d)	25.64 (d)	25.67 (d)	
3''-CH <sub>3</sub>	22.38 (q×2)	22.36 (q×2)	22.42 (q×2)	

Values in (δ) ppm.

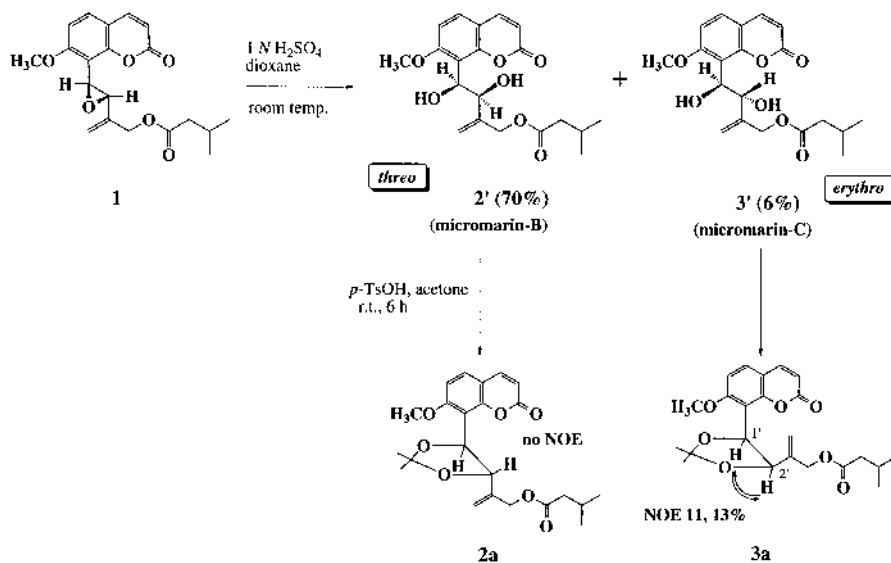
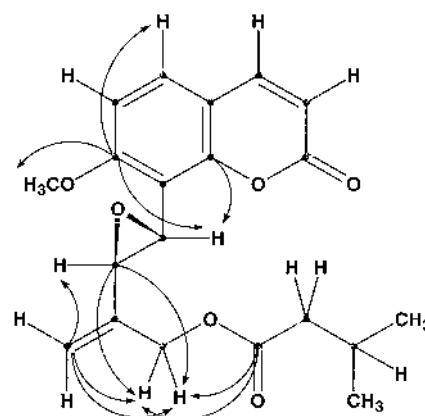


Chart 3

Fig. 1. C–H Long-Range Correlations in the HMBC Spectrum of Micromarin-A (1) in CDCl<sub>3</sub>

**Structure of Micromarin-G (5)** Micromarin-G (5) was obtained as a colorless oil, C<sub>15</sub>H<sub>14</sub>O<sub>4</sub>. The presence of a 7-methoxy-8-substituted coumarin nucleus was also suggested by the UV, IR, and <sup>1</sup>H-NMR spectra (see Table 1 and Experimental). In the <sup>1</sup>H-NMR spectrum, there were signals assignable to a Z-oriented disubstituted double bond [ $\delta$  6.42 (H-1'), 6.41 (H-2') (each 1H, d,  $J=12.2$  Hz)], an *exo*-methylene group [ $\delta$  4.87, 4.72 (each 1H, d,  $J=1.7$  Hz, H-4')], and a methylene [ $\delta$  4.22 (2H, s, H-5')] adjacent to a hydroxyl group. Based on the aforementioned results, together with mass fragment ions at  $m/z$  227 (M<sup>+</sup>·CH<sub>2</sub>-OH)<sup>+</sup> and 175 (M<sup>+</sup>·CH=CH(=CH<sub>2</sub>)-CH<sub>2</sub>-OH)<sup>+</sup> in the EI-MS, the structure of micromarin-G was concluded to be 5.

**Structure of Micromarin-H (6)** Micromarin-H (6) was obtained as a pale yellow oil. The molecular formula C<sub>15</sub>H<sub>14</sub>O<sub>4</sub> was found to be the same as that of 5 by HR-MS. The presence of the side-chain [(*E*)-CH=CH-C(=CH<sub>2</sub>)-CH<sub>2</sub>OH] at C-8 was suggested by the following spectral data: observations of 1) *exo*-methylene proton signals [ $\delta$  5.41, 5.36 (each 1H, d,  $J=1.5$  Hz, H-4')] and a 2H-singlet [ $\delta$  4.53 (H-5')] adjacent to a hydroxyl group, 2) AB-type signals at  $\delta$  7.44 and 7.00 (each 1H, d) having a large coupling constant ( $J=17.1$  Hz) in the <sup>1</sup>H-NMR spectrum, 3) mass fragment ions at  $m/z$  227 (M<sup>+</sup>·CH<sub>2</sub>-OH)<sup>+</sup> and 175 (M<sup>+</sup>·CH=CH(=CH<sub>2</sub>)-CH<sub>2</sub>-OH)<sup>+</sup> in the EI-MS. Based on these results, we assigned the structure 6 to micromarin-H.

Known coumarins isolated from the same plant material were characterised as micromelin (7),<sup>5,7,8</sup> murralonginol isovalerate (8),<sup>11,12</sup> microminutinin (9),<sup>3</sup> 6-methoxymicrominutinin (10),<sup>3</sup> microminutin (11),<sup>5</sup> and murrangatin (12)<sup>11,12</sup> by comparisons of <sup>1</sup>H-NMR and IR spectra with spectroscopic data reported in the literature.<sup>3,5,7,8,11,12</sup>

## Experimental

<sup>1</sup>H- and <sup>13</sup>C-NMR, H-H correlation spectroscopy (COSY), NOE, and HMBC ( $J=8$  Hz) spectra were recorded on an A-400 or A-600 (JEOL) spectrometer in CDCl<sub>3</sub>, unless otherwise stated. Chemical shifts are shown in  $\delta$  values (ppm) with tetramethylsilane (TMS) as an internal reference. MS were recorded on an M-80 (Hitachi), HX-110 (JEOL), or JMS-700 (JEOL) spectrometer having a direct inlet system. UV spectra were recorded on a V-550 UV/VIS spectrophotometer (JASCO) in MeOH, IR spectra on a FT/IR-230 (JASCO) in CHCl<sub>3</sub>, and optical rotations on a DIP-370 (JASCO) in CHCl<sub>3</sub> at 25 °C. Preparative TLC was carried out on Kieselgel 60 F<sub>254</sub> (Merck).

**Plant Material** Stems of *Micromelum minutum* WIGHT et ARN (Rutaceae) were collected in Nakorn-Rachasima province, Thailand, in April, 1996. Voucher specimens have been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University. The plant materials were identified by Dr. Nijisiri Ruangrunsi and compared with herbarium specimens at the Royal Forest Department, Ministry of Agriculture and Cooperative, Bangkok.

**Extraction and Isolation** Dried stems of the plant (160 g) were extracted with acetone at room temperature. The acetone extract (2.36 g) was subjected to silica-gel column chromatography eluting with hexane and hexane-acetone (7:3, 3:2, 1:1, 1:4), successively, to give 6 fractions. Each fraction was further subjected to silica-gel column chromatography and preparative TLC with appropriate combinations of hexane, CH<sub>2</sub>Cl<sub>2</sub>, iso-Pr<sub>2</sub>O, benzene, CHCl<sub>3</sub>, EtOAc, acetone, and MeOH as developing solvents to give six new coumarins along with six known coumarins, as stated below. The hexane-acetone (7:3) eluate gave micromarin-A (1) (57.9 mg), micromelin (7) (28.8 mg), murralonginol isovalerate (8) (0.4 mg), microminutinin (9) (47.1 mg), 6-methoxymicrominutinin (10) (9.7 mg), micromarin-F (4) (10.7 mg), and micromarin-G (5) (1.1 mg). The hexane-acetone (1:1) eluate gave microminutin (11) (105.1 mg), micromarin-H (6) (1.0 mg), micromarin-C (3) (1.2 mg), and murrangatin (12) (0.5 mg). The hexane-acetone (1:4) eluate gave micromarin-B (2) (7.5 mg).

Micromarin-A (1): Colorless powder. [ $\alpha$ ]<sub>D</sub><sup>20</sup> +3.44° ( $c=0.16$ , CHCl<sub>3</sub>). UV

$\lambda_{\max}$  (log  $\epsilon$ ) nm: 209 (4.82), 256 (4.14), 267 (4.03), 321 (4.60). IR  $\nu_{\max}$  cm<sup>-1</sup>: 1732, 1606. EI-MS  $m/z$  (%): 358 (M<sup>+</sup>, 5), 274 (41), 257 (40), 256 (78), 245 (23), 229 (33), 213 (38), 211 (29), 205 (31), 203 (33), 190 (100), 185 (57), 175 (15). HR-MS Calcd for C<sub>20</sub>H<sub>22</sub>O<sub>6</sub>: 358.1414. Found: 358.1385. Differential NOE: irradiation at  $\delta$  3.96 (7-OCH<sub>3</sub>) gave 15% enhancement at  $\delta$  6.87 (H-6).

Micromarin-B (2): Pale yellow oil. [ $\alpha$ ]<sub>D</sub><sup>20</sup> -1.64° ( $c=0.22$ , CHCl<sub>3</sub>). UV  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 207 (4.74), 247 (3.95), 258 (3.93), 322 (4.42). IR  $\nu_{\max}$  cm<sup>-1</sup>: 3545 (br), 1732, 1606. EI-MS  $m/z$  (%): 257 (52), 206 (100), 205 (99), 189 (11), 175 (27). FAB-MS  $m/z$ : 377 (M+H)<sup>+</sup>. HR-FAB-MS Calcd for C<sub>20</sub>H<sub>25</sub>O<sub>7</sub>: 377.1600. Found: 377.1591. Differential NOE: irradiation at  $\delta$  3.95 (7-OCH<sub>3</sub>) gave 26% enhancement at  $\delta$  6.88 (H-6). HMBC C-H correlations: C-2→H-3, H-4; C-4→H-5; C-4a→H-3, H-6; C-5→H-4; C-7→H-5, H-6, 7-OCH<sub>3</sub>; C-8→H-6, H-2'; C-8a→H-4, H-5; C-1'→H-2'; C-2'→H-4', H-5'; C-3'→H-2', H-4', H-5'; C-4'→H-5'; C-5'→H-2', H-4'; C-1''→H-5', H-2''; C-2''→H-3'', 3''-CH<sub>3</sub>; C-3''→H-2'', 3''-CH<sub>3</sub>; 3''-CH<sub>3</sub>→H-2'', H-3''.

Micromarin-C (3): Pale yellow oil. [ $\alpha$ ]<sub>D</sub><sup>20</sup> -8.11° ( $c=0.09$ , CHCl<sub>3</sub>). UV  $\lambda_{\max}$  nm: 205, 248, 257, 321. IR  $\nu_{\max}$  cm<sup>-1</sup>: 3568 (br), 1732, 1608. EI-MS  $m/z$  (%): 257 (3), 205 (100), 190 (7), 175 (13). FAB-MS  $m/z$ : 377 (M+H)<sup>+</sup>. HR-FAB-MS Calcd for C<sub>20</sub>H<sub>25</sub>O<sub>7</sub>: 377.1600. Found: 377.1580. Differential NOE: irradiation at  $\delta$  3.97 (7-OCH<sub>3</sub>) gave 19% enhancement at  $\delta$  6.90 (H-6). HMBC C-H correlations: C-2→H-3, H-4; C-4→H-5; C-4a→H-3, H-6; C-5→H-4; C-7→H-5, H-6, H-1', 7-OCH<sub>3</sub>; C-8→H-6, H-1', H-2'; C-8a→H-4, H-5, H-1'; C-1'→H-2', 1'-OH, 2'-OH; C-2'→H-1', 2'-OH, H-4', H-5'; C-3'→H-2', 2'-OH, H-4', H-5'; C-4'→H-5'; C-5'→H-2', H-4'; C-1''→H-5', H-2''; C-2''→H-3'', 3''-CH<sub>3</sub>; C-3''→H-2'', 3''-CH<sub>3</sub>; 3''-CH<sub>3</sub>→H-2'', H-3''.

**Treatment of Micromarin-A (1) with 1 N H<sub>2</sub>SO<sub>4</sub>** A solution of micromarin-A (1) (9.8 mg) in dioxane (2.5 ml) and 1 N H<sub>2</sub>SO<sub>4</sub> (2.5 ml) was stirred for 1.5 h at room temperature. The reaction mixture was treated in the usual manner and the residue was subjected to preparative TLC (MeOH-CHCl<sub>3</sub>, 1:4) to yield glycols 2' (7.2 mg) and 3' (0.6 mg). Glycols 2' and 3' were found to be identical with natural 2 and 3, respectively, by IR, <sup>1</sup>H-NMR, and co-TLC comparisons.

**Micromarin-B Acetonide (2a)** A solution of micromarin-B (2) (2.1 mg) in acetone (1.0 ml) and a catalytic amount of *p*-TsOH were stirred for 20 min at room temperature. The mixture was then neutralized with Et<sub>3</sub>N and the solvent was evaporated under reduced pressure. The residue was subjected to silica-gel preparative TLC (MeOH-CHCl<sub>3</sub>, 1:4) to yield compound (2a) (2.0 mg) as a colorless oil. UV  $\lambda_{\max}$  nm: 206, 219 (sh), 249, 258, 321. IR  $\nu_{\max}$  cm<sup>-1</sup>: 1732, 1608. EI-MS  $m/z$  (%): 416 (M<sup>+</sup>, 1), 401 (3), 257 (24), 246 (47), 212 (17), 205 (22), 189 (27), 160 (21), 127 (26), 110 (100). <sup>1</sup>H-NMR  $\delta$ : 7.61 (1H, d,  $J=9.5$  Hz, H-4), 7.42 (1H, d,  $J=8.4$  Hz, H-5), 6.88 (1H, d,  $J=8.4$  Hz, H-6), 6.27 (1H, d,  $J=9.5$  Hz, H-3), 5.57 (1H, d,  $J=8.8$  Hz, H-1'), 5.31 (1H, s, H-4'), 5.21 (1H, d,  $J=8.8$  Hz, H-2'), 5.19 (1H, s, H-4'), 4.56 (1H, d,  $J=13.9$  Hz, H-5'), 4.54 (1H, d,  $J=13.9$  Hz, H-5'), 3.93 (3H, s, 7-OCH<sub>3</sub>), 2.06 (2H, d,  $J=7.3$  Hz, H-2''), 2.00 (1H, m, H-3''), 1.71 (3H, s, CH<sub>3</sub>), 1.54 (3H, s, CH<sub>3</sub>), 0.88 (6H, d,  $J=6.6$  Hz, 3''-CH<sub>3</sub>).

**Micromarin-C Acetonide (3a)** Micromarin-C (3) (0.6 mg) was treated with acetone and *p*-TsOH under the same conditions as for 2 to yield compound (3a) (0.5 mg) as a colorless oil. UV  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 204, 224 (sh), 249, 259, 321. IR  $\nu_{\max}$  cm<sup>-1</sup>: 1732, 1606. EI-MS  $m/z$  (%): 416 (M<sup>+</sup>, 1), 401 (3), 258 (3), 246 (53), 212 (19), 205 (28), 189, (34), 160 (26), 127 (36), 110 (100). <sup>1</sup>H-NMR  $\delta$ : 7.56 (1H, d,  $J=9.5$  Hz, H-4), 7.35 (1H, d,  $J=8.4$  Hz, H-5), 6.80 (1H, d,  $J=8.4$  Hz, H-6), 6.22 (1H, d,  $J=9.5$  Hz, H-3), 6.13 (1H, d,  $J=8.8$  Hz, H-1'), 5.41 (1H, br s, H-4'), 5.13 (1H, d,  $J=8.4$  Hz, H-2'), 4.95 (1H, s, H-4'), 4.19 (1H, d,  $J=13.2$  Hz, H-5'), 4.11 (1H, d,  $J=13.2$  Hz, H-5'), 3.88 (3H, s, 7-OCH<sub>3</sub>), 2.11 (2H, d,  $J=7.3$  Hz, H-2''), 2.01 (1H, m, H-3''), 1.81 (3H, s, CH<sub>3</sub>), 1.50 (3H, s, CH<sub>3</sub>), 0.89 (6H, d,  $J=6.6$  Hz, 3''-CH<sub>3</sub>). Differential NOE: irradiation at  $\delta$  6.13 (H-1') gave 13% enhancement at  $\delta$  5.13 (H-2'); irradiation at  $\delta$  5.13 (H-2') gave 11% enhancement at  $\delta$  6.13 (H-1').

Micromarin-F (4): Colorless oil. UV  $\lambda_{\max}$  (log  $\epsilon$ ) nm: 206 (4.61), 220 (sh) (4.09), 249 (3.68), 257 (3.72), 322 (4.18). IR  $\nu_{\max}$  cm<sup>-1</sup>: 3535 (br), 1720, 1608. EI-MS  $m/z$  (%): 260 (M<sup>+</sup>, 27), 242 (17), 229 (12), 211 (24), 190 (58), 175 (19), 167 (69), 151 (100). HR-MS Calcd for C<sub>13</sub>H<sub>10</sub>O<sub>4</sub>: 260.1047. Found: 260.1030. Differential NOE: irradiation at  $\delta$  3.92 (7-OCH<sub>3</sub>) gave 15% enhancement at  $\delta$  6.84 (H-6); irradiation at  $\delta$  3.99 (H-5') gave 9% enhancement at  $\delta$  5.50 (H-2') and 4% enhancement at  $\delta$  1.89 (H-4'); irradiation at  $\delta$  3.60 (H-1') gave 7% enhancement at  $\delta$  5.50 (H-2') and 8% enhancement at  $\delta$  1.89 (H-4'); irradiation at  $\delta$  1.89 (H-4') gave 4% enhancement at  $\delta$  3.60 (H-1') and 4% enhancement at  $\delta$  3.99 (H-5'). HMBC C-H correlations: C-2→H-3, H-4; C-3→H-4; C-4→H-5; C-4a→H-3, H-6; C-5→H-4; C-7→H-5, H-1', 7-OCH<sub>3</sub>; C-8→H-6, H-1', H-2'; C-8a→H-4, H-5, H-1'; C-1'→H-2'; C-2'→H-1', H-4', H-5'; C-3'→H-1', H-4', H-5'; C-4'→H-2', H-5'; C-5'→H-2', H-4'.

Micromarin-G (5); Colorless oil. UV  $\lambda_{\max}$  nm: 205, 264, 276, 315. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3508 (br), 1724, 1601. EI-MS  $m/z$  (%): 258 ( $M^+$ , 100), 227 (23), 226 (40), 213 (25), 197 (46), 185 (25), 175 (16). HR-MS Calcd for  $C_{15}H_{14}O_4$ : 258.0891. Found: 258.0902. Differential NOE: irradiation at  $\delta$  3.95 (7-OCH<sub>3</sub>) gave 10% enhancement at  $\delta$  6.88 (H-6).

Micromarin-H (6); Pale yellow oil. UV  $\lambda_{\max}$  nm: 205, 259, 274, 301, 315. IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3534 (br), 1722, 1599. EI-MS  $m/z$  (%): 258 ( $M^+$ , 100), 227 (19), 222 (30), 213 (24), 197 (34), 185 (24), 175 (17). HR-MS Calcd for  $C_{15}H_{14}O_4$ : 258.0891. Found: 258.0909. Differential NOE: irradiation at  $\delta$  3.97 (7-OCH<sub>3</sub>) gave 17% enhancement at  $\delta$  6.87 (H-6).

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