

### C3\_C0035: CHEMICAL CONSTITUENTS FROM THE LEAVES OF *Miliusa longipes* (ANNONACEAE)

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**Abstract:** Phytochemical investigation of the leaves of *Miliusa longipes* (Annonaceae) led to the isolation and identification of six known compounds including two geranylated homogentisic acid derivatives, (-)-miliusane XIX (**1**) and (+)-miliusol (**2**), two flavonoids, 5,4'-dihydroxy-3,7-dimethoxyflavone (kumatakenin) (**3**) and 5,3',4'-trihydroxy-3,7-dimethoxyflavone (**4**), stigmast-5-en-3-*O*- $\beta$ -glucoside (**5**) and one sesquiterpenoid, isointermedeol (**6**). Their chemical structures were determined by analysis of their spectroscopic data as well as by data comparison with those reported in the literature.

**Introduction:** *Miliusa longipes* belongs to the Annonaceae family. It is a small shrub, distributed in the South and Southeast regions of Thailand. There has been no report on the chemical constituents of this plant. However, many types of secondary metabolites have been isolated from *Miliusa* genus including alkaloids,<sup>1-4</sup> neolignans,<sup>4-6</sup> lignans,<sup>6</sup> flavonoids,<sup>4,7-9</sup> styryl lactones,<sup>8</sup> geranylated homogentisic acid derivatives,<sup>7-11</sup> acetogenins,<sup>12</sup> and other aliphatic and aromatic compounds.<sup>4,9,13</sup> Several of these chemical constituents displayed cytotoxic,<sup>5-6,10-12</sup> anti-herpetic activity,<sup>5-6</sup> and antibacterial.<sup>12</sup> Herein, the isolation and structural elucidation of compounds (**1-6**) are described from an EtOAc extract of the leaves of *M. longipes*.

#### Methodology:

**General Experimental Procedures:** Melting points were measured with a Buchi melting point B-540 apparatus and are uncorrected. Optical rotations were measured on A.KRÜSS optronic PS8000 polarimeter. UV-Vis spectra were taken in MeOH solution on a SPECORD® 210 PLUS analytik Jena spectrophotometer. IR spectra were recorded with a Shimadzu FTIR-8900 IR spectrophotometer. NMR spectra were recorded in CDCl<sub>3</sub> or pyridine-d<sub>5</sub> with TMS as the internal reference on a Bruker AVANCE400 spectrometer (<sup>1</sup>H at 400 MHz and <sup>13</sup>C at 100 MHz). Vacuum liquid chromatography (VLC) was carried out on silica gel 60H (Merck, 5-40  $\mu$ m) and RP-18 (Merck, 15-25  $\mu$ m). TLC was performed on precoated silica gel 60 F<sub>254</sub> plates (Merck) and RP-18 F<sub>254S</sub> plates (Merck). Spots were detected by UV or sprayed with 1% Ce(SO<sub>4</sub>)<sub>2</sub> in 10% aq. H<sub>2</sub>SO<sub>4</sub>. Commercial grade solvents were distilled prior to use.

**Plant material:** The leaves of *M. longipes* were collected from Surat Thani Province, Thailand, in March 2012. This plant was identified by Dr. Piya Chalermglin, Thailand Institute of Scientific and Technological Research, Thailand. A voucher specimen (PKRU2013001) was deposited at the Laboratory of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University, Phuket, Thailand.

**Extraction and Isolation:** The air-dried leaves of *M. longipes* (650.0 g) were extracted with EtOH (3 x 10 L) for a week at room temperature. The EtOH extract was evaporated to dryness under reduced pressure to give a crude extract (110.0 g) which was partitioned between EtOAc and H<sub>2</sub>O. The EtOAc layer was evaporated to dryness under reduced pressure to give a crude EtOAc extract (41.0 g). The crude EtOAc extract was subjected to VLC over silica gel 60H using a gradient of EtOAc and hexane to yield 13 fractions (F1-F13). Fraction F3 (2.1 g) was separated by VLC over silica gel 60H using a gradient of

EtOAc and hexane to afford 9 subfractions (F3<sub>a</sub>-F3<sub>i</sub>). Subfraction F3<sub>c</sub> (325.9 mg) was separated by VLC over silica gel 60H using a gradient of EtOAc and hexane to give 4 subfractions (F3<sub>ca</sub>-F3<sub>cd</sub>). Subfraction F3<sub>cb</sub> was purified by VLC over silica gel 60H using a gradient of CH<sub>2</sub>Cl<sub>2</sub> and hexane to afford **6** (2.9 mg). Fraction F6 (1.9 g) was separated by VLC over silica gel 60H using a gradient of EtOAc and hexane to provide 11 subfractions (F6<sub>a</sub>-F6<sub>k</sub>). Subfraction F6<sub>d</sub> was purified by VLC over silica gel RP-18 using a gradient of H<sub>2</sub>O and MeOH to give **1** (37.2 mg). Fraction F10 (14.1 g) was recrystallized from MeOH to afford 2 subfractions (F10<sub>a</sub>-F10<sub>b</sub>). Subfraction F10<sub>b</sub> (2.1 g) was separated by VLC over silica gel 60H using a gradient of EtOAc and CH<sub>2</sub>Cl<sub>2</sub> to give 3 subfractions (F10<sub>ba</sub>-F10<sub>bc</sub>). Subfraction F10<sub>bc</sub> (1.0 g) was purified by VLC on silica gel 60H using a gradient of acetone and CH<sub>2</sub>Cl<sub>2</sub> to afford **3** (230.0 mg) and **4** (180.0 mg). Subfraction F10<sub>c</sub> (11.5 g) was further purified by VLC over silica gel RP-18 using a gradient of H<sub>2</sub>O and MeOH to give **2** (306.4 mg). Fraction F12 (2.2 g) was recrystallized from MeOH to yield **5** (10.0 mg).

**Compound (1):** yellow viscous gum; C<sub>19</sub>H<sub>26</sub>O<sub>4</sub>; [ $\alpha$ ]<sub>D</sub><sup>25</sup> - 22.0° (*c* 5.40, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 274 (3.43) nm; IR (film)  $\nu_{\max}$  2926, 1736, 1686, 1437, 1360, 1267, 1180, 1013, 926 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1.

**Compound (2):** colorless viscous gum; C<sub>18</sub>H<sub>24</sub>O<sub>4</sub>; [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 69.9° (*c* 0.75, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 255 (4.01), 343 (3.09) nm; IR (film)  $\nu_{\max}$  3179, 1780, 1678, 1211, 982, 922, 829 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1.

**Compound (3):** yellow needles; C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>; m.p. 354-356 °C; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 268 (4.66), 351 (4.71) nm; IR (KBr)  $\nu_{\max}$  3240, 1663, 1601, 1580, 1497, 1375, 1344, 1286, 1169 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2.

**Compound (4):** yellow solid; C<sub>17</sub>H<sub>14</sub>O<sub>7</sub>; m.p. 241-243 °C; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 257 (4.64), 358 (4.58) nm; IR (KBr)  $\nu_{\max}$  3179, 3092, 1661, 1595, 1497, 1433, 1161, 1115 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2.

**Compound (5):** white solid; C<sub>35</sub>H<sub>60</sub>O<sub>6</sub>; [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 16.4° (*c* 0.02, pyridine); m.p. 290-293 °C; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 277 (3.58) nm; IR (ATR)  $\nu_{\max}$  3375, 2958, 2931, 2866, 1460, 1365, 1164, 1103, 1070 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 3.

**Compound (6):** white solid; C<sub>15</sub>H<sub>26</sub>O; [ $\alpha$ ]<sub>D</sub><sup>25</sup> - 69.2° (*c* 0.02, CHCl<sub>3</sub>); m.p. 82-84 °C; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 278 (3.18) nm; IR (KBr)  $\nu_{\max}$  3314, 2926, 2853, 1641, 1454, 1377, 1169, 881 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 4.

**Results and Discussion:** The air-dried leaves of *M. longipes* were extracted with EtOH and the extract was partitioned between EtOAc and H<sub>2</sub>O. Chromatographic separation of the EtOAc fraction led to the isolation of compounds **1-6** (Figure 1).

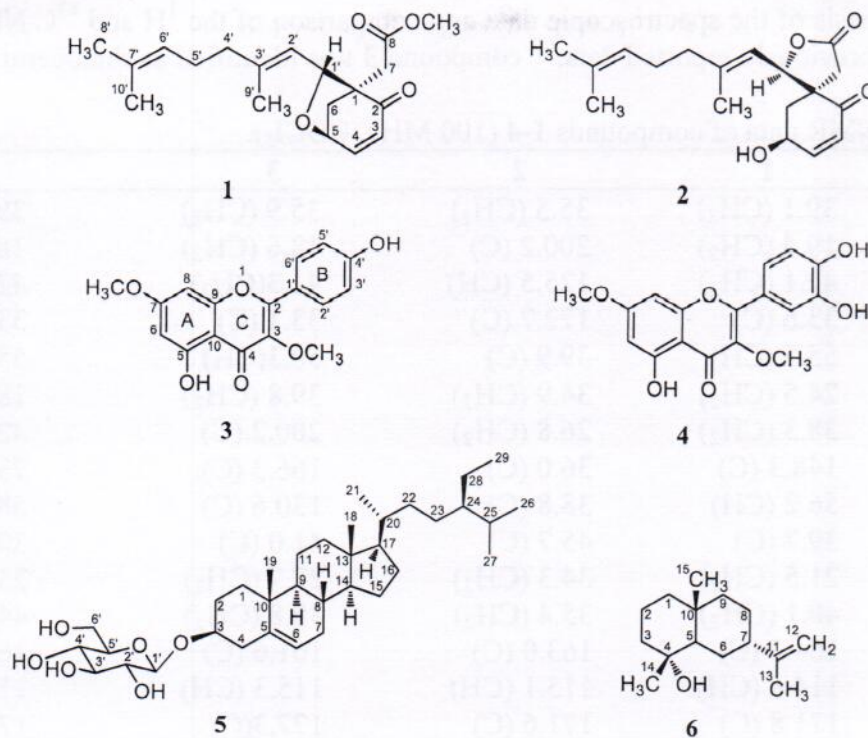


Figure 1. Structures of compounds 1-6.

Compound **1** was obtained as a yellow viscous gum with  $[\alpha]_D^{25} - 22.0^\circ$  ( $c$  5.40,  $\text{CHCl}_3$ ). The IR spectrum displayed absorption bands indicative of an  $\alpha,\beta$ -unsaturated ketone ( $1686\text{ cm}^{-1}$ ) and an ester carbonyl group ( $1736\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum (Table 1) showed signals for two olefinic protons of the  $\alpha,\beta$ -unsaturated ketone at  $\delta_{\text{H}}$  5.90 (1H, d,  $J = 9.6$  Hz, H-3) and 7.30 (1H, dd,  $J = 9.6, 5.6$  Hz, H-4), two aromatic protons of two trisubstituted alkenes at  $\delta_{\text{H}}$  4.56 (1H, br d,  $J = 12.4$  Hz, H-2') and 4.85 (1H, br t,  $J = 7.2$  Hz, H-6'), two oxymethine protons at  $\delta_{\text{H}}$  4.50 (1H, br t,  $J = 5.2$  Hz, H-5) and 4.54 (1H, d,  $J = 10.4$  Hz, H-1'), three vinyl methyl groups at  $\delta_{\text{H}}$  1.42 (3H, s,  $\text{CH}_3$ -8'), 1.53 (3H, s,  $\text{CH}_3$ -9') and 1.52 (3H, s,  $\text{CH}_3$ -10'), and a methoxy group at  $\delta_{\text{H}}$  3.50. The vinyl methyls together with the two trisubstituted double bonds suggested the presence of a geranyl moiety. The  $^{13}\text{C}$  NMR spectrum (Table 1) showed 19 carbons including an enone carbonyl at  $\delta_{\text{C}}$  198.8 (C-1), an ester carbon at  $\delta_{\text{C}}$  171.4 (C-8) and two secondary oxygenated carbons at  $\delta_{\text{C}}$  72.4 (C-5) and 79.1 (C-1'). The signals of the geranyl moiety appeared at  $\delta_{\text{C}}$  102.7 (C-2'), 143.0 (C-3'), 39.6 (C-4'), 26.0 (C-5'), 123.5 (C-6'), 131.5 (C-7'), 16.2 (C-8'), 25.5 (C-9') and 17.6 (C-10'). The DEPT and HMQC spectra enabled us to conclude that **1** had five quaternary, six methine, four methylene and four methyl carbons. The HMBC spectrum showed correlations between the oxymethine H-5 ( $\delta_{\text{H}}$  4.50) and C-1' ( $\delta_{\text{C}}$  79.1), which suggested the formation of a tetrahydrofuran ring system with an ether linkage between C-1' and C-5. The methoxy protons ( $\delta_{\text{H}}$  3.50) showed the same correlation to the carbonyl carbon (C-8,  $\delta_{\text{C}}$  171.4), suggesting the presence of a methyl ester moiety. These data indicated that **1** was a geranyl homogentisic acid derivative. The NOESY spectrum showed correlations of H-5 ( $\delta_{\text{H}}$  4.50) to H-4 ( $\delta_{\text{H}}$  7.30) and H-6 $\alpha$  ( $\delta_{\text{H}}$  2.67) and H-1' ( $\delta_{\text{H}}$  4.54) to H-6 $\beta$  ( $\delta_{\text{H}}$  2.06) and H<sub>3</sub>-9' ( $\delta_{\text{H}}$  1.53). Therefore, **1** was deduced as (-)-miliusane XIX or [7 $\alpha$ -(2,6-dimethyl-hepta-1,5-dienyl)-2-oxo-6 $\beta$ -oxa-bicyclo[3.2.1]oct-3-en-1-yl]acetic acid methyl ester by comparison of its physical and spectral data with those reported in the literature.<sup>11</sup>

Compound **2** was obtained as a colorless viscous gum with  $[\alpha]_D^{25} + 69.9^\circ$  ( $c$  0.75,  $\text{CHCl}_3$ ). The IR spectrum was similar to that of **1** except for an additional absorption band of a hydroxy group ( $3179\text{ cm}^{-1}$ ). The NMR spectroscopic data were similar to those of **1** except

for the absence of signals for the methoxy group at  $\delta_{\text{H}}$  3.50 and  $\delta_{\text{C}}$  51.4 in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, respectively. In addition, the oxymethine carbon (C-5) in **2** (Table 1) shifted approximately 9 ppm to upfield in comparison with that of **1**. The  $^3\text{J}$  HMBC correlation between H-1' ( $\delta_{\text{H}}$  5.16) and the ester carbonyl carbon at  $\delta_{\text{C}}$  175.8 established a spiro  $\gamma$ -lactone ring. The NOESY spectrum showed correlations of H-5 ( $\delta_{\text{H}}$  4.57) to H-4 ( $\delta_{\text{H}}$  6.91) and H-6 $\alpha$  ( $\delta_{\text{H}}$  2.20) and H-6 $\beta$  ( $\delta_{\text{H}}$  2.30) to H-7 $\beta$  ( $\delta_{\text{H}}$  3.15) and H-2' ( $\delta_{\text{H}}$  5.67). Accordingly, **2** was deduced as (+)-miliusol or 9 $\beta$ -hydroxy-1 $\beta$ -(*E*-2,6-dimethyl-hepta-1,5-dienyl)-2-oxaspiro[4.5]dec-7-ene-3,6-dione by comparison of its physical and spectral data with those reported in the literature.<sup>11</sup>

**Table 1.** The  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of Compounds **1** and **2** in  $\text{CDCl}_3$ .

Position	1		2	
	$\delta_{\text{H}}^{\text{b}}$ mult. ( <i>J</i> in Hz)	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{H}}^{\text{b}}$ mult. ( <i>J</i> in Hz)	$\delta_{\text{C}}^{\text{a}}$
1	-	44.8	-	52.4
2	-	198.8	-	196.6
3	5.90, d (9.6)	130.6	5.96, dd (10.4, 0.8)	128.7
4	7.30, dd (9.6, 5.6)	153.4	6.91, dd (10.4, 4.0)	149.6
5	4.50, br t (5.2)	72.4	4.57, br qn <sup>a</sup> (4.0)	63.3
6	6 $\alpha$ : 2.67, d (11.2) 6 $\beta$ : 2.06, dd (11.2, 5.5)	44.7	6 $\alpha$ : 2.20, dd (14.0, 5.2) 6 $\beta$ : 2.30, dd (14.0, 4.8)	39.4
7	7a: 2.82, d (16.4) 7b: 2.10, d (16.8)	34.8	7 $\alpha$ : 2.36, d (17.2) 7 $\beta$ : 3.15, d (17.2)	38.3
8	-	171.4	-	175.8
1'	4.54, d (10.4)	79.1	5.16, d (10.0)	82.6
2'	4.56, br d (12.4)	120.7	5.67, d (10.0)	117.9
3'	-	143.0	-	145.4
4'	1.81, m	39.6	1.99, m	39.6
5'	1.89, m	26.0	2.04, m	25.6
6'	4.85, br t (7.2)	123.5	4.99, br t (1.2)	123.2
7'	-	131.5	-	131.9
8'	1.42, s	16.2	1.71, d (1.2)	26.0
9'	1.53, s	25.5	1.58, s	17.7
10'	1.52, s	17.6	1.67, s	16.9
3-OCH <sub>3</sub>	3.50, s	51.4	-	-
5-OH	-	-	2.78, br s	-

<sup>a</sup>qn represents quintet.

Compound **3** was obtained as yellow needles. Its IR spectrum showed absorption bands of hydroxy ( $3240\text{ cm}^{-1}$ ), carbonyl ( $1663\text{ cm}^{-1}$ ) and aromatic ( $1497\text{-}1601\text{ cm}^{-1}$ ) groups. The presence of an aromatic ring was supported by UV absorption bands at  $\lambda_{\text{max}}$  268 and 351 nm. The  $^{13}\text{C}$  NMR spectrum (Table 2) showed 17 signals for the aromatic carbons of a flavone, including six oxygenated carbons, together with resonances for two methoxy groups and a carbonyl group. The  $^1\text{H}$  NMR spectrum (Table 2) revealed the presence of two *meta*-coupled aromatic protons of A ring at  $\delta_{\text{H}}$  6.60 (1H, d, *J* = 2.0 Hz, H-6) and 6.69 (1H, d, *J* = 2.0 Hz, H-8), two *ortho*-coupled aromatic protons of a *para* disubstituted B ring at  $\delta_{\text{H}}$  7.31 (2H, dd, *J* = 8.8 Hz, H-3'/5') and 8.21 (2H, dd, *J* = 8.4 Hz, H-2'/6'), and a chelated hydroxy proton at  $\delta_{\text{H}}$  13.34. The HMBC correlation of the methoxy group at  $\delta_{\text{H}}$  3.80 with C-7 ( $\delta_{\text{C}}$  165.6) and that of the remaining methoxy group at  $\delta_{\text{H}}$  3.99 with C-3 ( $\delta_{\text{C}}$  138.6) indicated the location of these methoxy groups at C-7 and C-3, respectively. The chelated hydroxy proton

( $\delta_{\text{H}}$  13.34) showed the  $^3J$  HMBC correlations with C-10 and C-6 and a  $^2J$  HMBC correlation with C-5. Hence this hydroxy group was attached at C-5. Compound **3** was identified as 5,4'-dihydroxy-3,7-dimethoxyflavone or kumatakenin by comparison of its physical and spectral data with those reported in the literature.<sup>14</sup>

**Table 2.** The  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of Compounds **3** and **4** in Pyridine- $d_5$ .

Position	3		4	
	$\delta_{\text{H}}$ mult. ( $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ mult. ( $J$ in Hz)	$\delta_{\text{C}}$
1	-	-	-	-
2	-	156.4	-	156.6
3	-	138.6	-	138.6
4	-	178.8	-	178.8
5	-	162.2	-	162.1
6	6.60, d (2.0)	98.1	6.47, d (2.4)	98.0
7	-	165.6	-	165.5
8	6.69, d (2.0)	92.2	6.51, d (2.4)	92.1
9	-	156.9	-	156.8
10	-	106.1	-	106.1
1'	-	121.3	-	121.8
2'	8.21, d (8.4)	130.8	8.15, d (2.0)	116.5
3'	7.31, d (8.8)	116.3	-	147.0
4'	-	161.7	-	150.6
5'	7.31, d (8.8)	116.3	7.35, d (8.4)	116.4
6'	8.21, d (8.4)	130.8	7.79, dd (8.4, 2.0)	121.4
3-OCH <sub>3</sub>	3.99, s	59.7	3.88, s	59.6
7-OCH <sub>3</sub>	3.80, s	55.7	3.76, s	55.7
5-OH	13.34, s	-	13.26, s	-

Compound **4** was obtained as a yellow solid. The UV, IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic features were similar to those of **3**, implying that **4** was also a flavone derivative. The difference was the structure of B ring. The  $^1\text{H}$  NMR spectrum of the B ring (Table 2) showed signals for a 1,3,4-trisubstituted benzene [ $\delta_{\text{H}}$  8.15 (1H, d,  $J = 2.0$  Hz), 7.35 (1H, d,  $J = 8.4$  Hz), and 7.79 (1H, dd,  $J = 8.4, 2.0$  Hz)] instead of signals for the 1,4-disubstituted benzene of B ring in **3**. The aromatic proton at  $\delta_{\text{H}}$  8.15 showed the  $^3J$  HMBC correlations with C-2, C-4' and C-6', thus it was assigned as H-2'. Two other aromatic protons at  $\delta_{\text{H}}$  7.35 and 7.79 were then attributed to H-5' and H-6', respectively, on the basis of their multiplicities and coupling constants. The substituents at C-3' and C-4' were identified as two hydroxy groups on the basis of their chemical shifts. Consequently, **4** was assigned as 5,3',4'-trihydroxy-3,7-dimethoxyflavone by comparison of its physical and spectral data with those reported in the literature.<sup>15</sup>

Compound **5** was obtained as a white solid with  $[\alpha]_{\text{D}}^{25} + 16.4^\circ$  ( $c$  0.02, pyridine). The IR spectrum showed an absorption band for a hydroxy group ( $3476\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum showed signals for two tertiary methyl groups at  $\delta_{\text{H}}$  0.67 (3H, s, CH<sub>3</sub>-18) and 0.94 (3H, s, CH<sub>3</sub>-19), three tertiary methyl groups at  $\delta_{\text{H}}$  0.91 (3H, d,  $J = 7.6$  Hz, CH<sub>3</sub>-21), 1.01 (3H, d,  $J = 6.4$  Hz, CH<sub>3</sub>-26) and 0.99 (3H, d,  $J = 6.4$  Hz, CH<sub>3</sub>-27), one primary methyl group at  $\delta_{\text{H}}$  0.87 (3H, d,  $J = 7.6$  Hz, CH<sub>3</sub>-29), an olefinic proton of a trisubstituted alkene at  $\delta_{\text{H}}$  5.37 (1H, br t,  $J = 2.5$  Hz) and an oxymethine proton at  $\delta_{\text{H}}$  3.98 (1H, m, H-3). These results indicated that **5** was a 3-hydroxysterol derivative. Moreover, the  $^1\text{H}$  NMR spectrum exhibited

signals of a sugar moieties: one anomeric proton at  $\delta_{\text{H}}$  5.09 (1H, d,  $J = 7.6$  Hz, H-1'), 4.10 (1H, dd,  $J = 8.0, 7.6$  Hz, H-2'), 4.33 (1H, t,  $J = 6.8$  Hz, H-3'), 4.30 (1H, t,  $J = 8.8$  Hz, H-4'), 3.96 (1H, m, H-5') and 4.55 (1H, dd,  $J = 11.6, 4.8$  Hz, H-6'a), 4.60 (1H, d,  $J = 11.6$  Hz, H-6'b). The anomeric proton signal appeared as doublet with the coupling constant of 7.6 Hz, suggesting an  $\beta$ -form. These  $^1\text{H}$  NMR data together with 35 carbon resonances in  $^{13}\text{C}$  NMR (Table 3) and DEPT spectra corresponding to six methyls, eleven methylenes, nine methines and three quaternary carbons together with five methine resonances at  $\delta_{\text{C}}$  102.3, 78.4, 77.9, 75.1 and 71.5 and one methylene resonance at  $\delta_{\text{C}}$  62.6 of the  $\beta$ -D-glucopyranoside suggested that **5** was a stigmast-5-ene glucoside derivative.<sup>16</sup>

**Table 3.** The  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of Compound **5** in Pyridine- $d_5$ .

Position	$\delta_{\text{H}}$ mult. ( $J$ in Hz)	$\delta_{\text{C}}$	HMBC
1	1.00, m/ 1.72, m	37.3	2, 3, 5, 10
2	2.14, m/ 1.75, m	30.0	1, 10
3	3.98, m	78.3	1', 10
4	2.76, m/ 2.50, m	39.1	2, 5, 10
5	-	140.7	-
6	5.37, br t (2.5)	121.7	7, 8
7	1.54, m/ 1.93, m	32.0	5, 6, 9
8	1.38, m	31.8	9, 10, 13
9	0.86, m	50.1	19
10	-	36.7	-
11	1.42, m	21.1	8, 9, 13
12	1.99, m/ 1.12, m	39.7	9, 14, 18
13	-	42.3	-
14	0.92, m	56.6	6, 9, 18
15	1.05, m/ 1.57, m	24.3	8, 13, 14, 17
16	1.88, m	28.3	13, 17
17	1.10, m	56.0	12, 13, 14, 18, 20, 22
18	0.67, s	11.8	12, 13, 14, 17
19	0.94, s	19.8	5, 9, 10
20	1.41, m	36.2	13, 17
21	0.91, d (7.6)	19.2	22
22	1.09, m	34.0	17, 21
23	1.25, m	26.1	20, 24, 25
24	1.02, m	45.8	22, 26, 27, 29
25	1.68, m	29.2	23, 24, 26, 27, 28
26	1.01, d (6.4)	19.0	25
27	0.99, d (6.4)	18.8	25
28	1.32, m	23.2	23, 24, 25, 29
29	0.87, d (7.6)	11.9	24
1'	5.09, d (7.6)	102.3	5'
2'	4.10, dd (8.0, 7.6)	75.1	1', 3'
3'	4.33, t (6.8)	78.4	4'
4'	4.30, t (8.8)	71.5	2', 3', 6'
5'	3.96, m	77.9	1'
6'a	4.55, dd (11.6, 4.8)	62.6	3', 4', 5'
6'b	4.60, d (11.6)		4'

The attachment of the sugar chain at C-3 of stigmasterol was determined by the HMBC correlation from the glucose-H-1' to C-3 of stigmasterol. The NOESY correlations of H-1' ( $\delta_H$  5.09), H-3' ( $\delta_H$  4.33) and H-5' ( $\delta_H$  3.96) is a characteristic of  $\beta$ -glucopyranosyl unit. Therefore, **5** was identified as stigmasterol-5-en-3-O- $\beta$ -glucoside by comparison of its physical and spectral data with those reported in the literature.<sup>17</sup>

Compound **6** was isolated as a white solid with  $[\alpha]_D^{25} - 69.2^\circ$  (*c* 0.02, CHCl<sub>3</sub>). It exhibited absorption bands for hydroxy (3314 cm<sup>-1</sup>) and double bond (1641 cm<sup>-1</sup>) functional groups in the IR spectrum. The <sup>13</sup>C NMR spectrum (Table 4) revealed the presence of 15 carbon signals, including three methyls, seven methylenes, two methines and three quaternary carbons including a downfield olefinic quaternary carbon at  $\delta_C$  150.7. These data indicated a sesquiterpenoid skeleton type eudesmans. The <sup>1</sup>H NMR spectral data (Table 4) revealed two signals of isopropenyl protons at  $\delta_H$  4.70 and 4.71 (H-12), a vinylic methyl singlet at  $\delta_H$  1.75 (H-13) and two methyl singlets at  $\delta_H$  1.12 (H-14) and 0.89 (H-15). In the HMBC spectrum, the geminal olefinic protons ( $\delta_H$  4.70, m, 4.71, m, H-12) showed correlations with C-7 ( $\delta_C$  46.3) and C-13 ( $\delta_C$  21.1) and H-5 ( $\delta_H$  1.27) showed cross peaks with C-6 ( $\delta_C$  26.0), C-7 ( $\delta_C$  46.3), C-10 ( $\delta_C$  34.6), and C-15 ( $\delta_C$  18.7). The NOESY spectrum displayed correlations between H-5 ( $\delta_H$  1.27) and H<sub>3</sub>-13 ( $\delta_H$  1.75), and H<sub>3</sub>-14 ( $\delta_H$  1.12) and H<sub>3</sub>-15 ( $\delta_H$  0.89). Thus, **6** was identified as isointermedeol by comparison of its physical and spectral data with those reported in the literature.<sup>18</sup>

**Table 4.** The <sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) data of Compound **6** in CDCl<sub>3</sub>.

Position	$\delta_H$ mult. (J in Hz)	$\delta_C$	HMBC
1	0.86, m/ 1.41, m	41.1	5
2	1.57, m/ 1.58, m	20.1	1, 3, 4, 10
3	1.38, m/ 1.81, m	43.4	14
4	-	72.3	-
5	1.27, m	54.9	6, 7, 10, 15
6	1.85, m/ 1.93, m	26.0	13
7	1.95, m	46.3	13
8	1.45, m/ 1.46, m	26.9	10
9	1.22, m/ 1.46, m	44.7	5, 7, 10, 15
10	-	34.6	-
11	-	150.7	-
12	4.70, m/ 4.71, m	108.1	7, 13
13	1.75, s	21.1	7, 11, 12
14	1.12, s	22.7	3, 4, 5
15	0.89, s	18.7	1, 5, 9, 10

**Conclusion:** Six known compounds (**1-6**) have been isolated from the EtOAc portion of the crude EtOH extract of the leaves of *M. longipes*. All structures were identified by analysis of their spectroscopic data and comparison with literature values. This is the first report on the isolation of **3-6** from the genus *Miliusa*.

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**Acknowledgements:** We are indebted to the office of the National Research Council of Thailand for financial support. We also thank Dr. Piya Chalermglin (Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand) for the identification of the plant. We are grateful to Department of Natural Products Chemistry, Faculty of Science and Technology, Phuket Rajabhat University for the provision of laboratory facilities and technical assistance and we also thank Mrs. Nareerat Banjongkarn, Phuket Rajabhat University for recording the NMR spectra.