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Letter

Chemical Constituents of *Millettia speciosa*

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ABSTRACT

Objective To study the chemical constituents from *Millettia speciosa*. **Methods** The compounds were isolated and purified by silica gel, Sephadex LH-20, ODS column chromatography, and recrystallization. The structures were identified using physicochemical and spectral data. **Results** Thirteen compounds were isolated from *M. speciosa* and identified as docosanoic acid **(1)**, tetracosane **(2)**, octadecane **(3)**, hexacosanoic acid **(4)**, β-sitosterol acetate **(5)**, β-sitosterol **(6)**, syringin **(7)**, maackiain **(8)**, formononetin **(9)**, ψ-baptigenin (**10**), rotundic acid **(11)**, pedunculoside **(12)**, and daucosterol **(13)**. **Conclusion** Compounds **5**, **7**, and **10**–**12** are obtained from this plant for the first time.

Key words

ψ-baptigenin; Leguminosae; *Millettia speciosa*; pedunculoside; rotundic acid; syringin

1. Introduction

Millettia speciosa Champ., belonging to Leguminosae, is a sub-shrub plant commonly known as a tonic and mainly growing in Guangdong and Guangxi provinces, China. Its roots are used to treat lumbago and strengthen the bones and muscles (Editorial Board of Chinese Materia Medica in Guangdong, 1991; State Administration of Editorial Board of Chinese Materia Medica, 1999). It was widely used as an edible plant with the effect of strengthening bones and muscles in local places, such as stewed chicken or soup. Previous phytochemical studies reported that the root extract of *M. speciosa* contains various constituents including alkaloid, oleanane-type triterpene saponin, flavonol, and phenolic glycosides (Uchiyama et al, 2003; Wang et al, 2008; Yin et al, 2008; Yin et al, 2010; Zong et al, 2009; Zhang *et al*, 2011), etc. In order to know more bioactive compounds and safe use of this herbal medicine, 13 compounds were isolated from petroleum ether, ethyl acetate, and *n*-butanol soluble parts of ethanol extract from the roots of *M. speciosa*. The compounds were identified as docosanoic acid (**1**), tetracosane (**2**), octadecane (**3**), hexacosanoic acid (**4**), β-sitosterol acetate (**5**), β-sitosterol (**6**), syringin (**7**), maackiain (**8**), formononetin (**9**), ψ-baptigenin (**10**), rotundic acid (**11**), pedunculoside (**12**), and daucosterol (**13**). Compounds **5**, **7**, and **10**–**12** were reported for the first time in this plant. In this paper, we report the isolation and structure elucidation of these compounds.

2. Materials and methods

2.1 General

TLC was performed on silica gel GF_{254} plates (Qingdao Marine Chemical Factory) and developed with 10% sulphuric acid in ethanol. Chromatography was carried out on silica gel (200–300 mesh, Qingdao Marine Chemical Factory), Sephadex LH-20 (Pharmacia), and RP-18 gel (50 μm, YMC, Japan) columns. Agilent 1100 Series LC/MSD Trap (USA Agilent Technologies) and Nicolet Impact 410 FT-IR Spectrometer were used; HR-ESI-MS was recorded on an APEX II spectrometer. Bruker AV-300 NMRS (Swiss, Bruker) was

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used. All the NMR data were obtained at room temperature (TMS as internal standard).

2.2 Plant material

The roots of *Millettia speciosa* Champ. were collected in Conghua, Guangdong province, China, in August 2010, and identified by Prof. Ping Ding. A voucher specimen was deposited at the herbarium of Research Laboratory of Chinese Medicinal Resources, Guangzhou University of Chinese Medicine.

2.3 Extraction and isolation

The dried sliced roots of *M. speciosa* (10 kg) were extracted with 95% EtOH (reflux, 2 h, three times). The combined extracts were concentrated in vacuum to yield a dark residue suspended in water and partitioned with ether, EtOAc, and *n*-BuOH. The ether extract (208 g) and EtOAc extract (250 g) were respectively subjected to column chromatography. The ether extract was re-chromatographed on a silica gel column eluted with gradient mixture of EtOEt-EtOAc (1:0 to 0:1) to yield compounds **1** (30 mg), **2** (32 mg), **3** (28 mg), and **4** (40 mg). The EtOAc extract was re-chromatographed on a silica gel column eluted with gradient mixture of $CHCl_3$-EtOAc (16:1 to 0:1) to give four fractions. Fr. 1 (3.47 g) was separated with silica gel column eluted with $CHCl_3$-EtOAc (1:1 to 0:1) to yield compounds **5** (53 mg) and **6** (20 mg). Fr. 2 (4.93 g) was subjected to repeated column chromatography on silica gel, eluted with $CHCl_3$-MeOH (12:1 to 0:1) to yield compounds **7** (43 mg), **8** (48 mg), **9** (64 mg), and **10** (37 mg). Fr. 3 (5.27 g) was subjected to repeated column chromatography on silica gel, eluted with EtOAc-MeOH (20:1 to 0:1) to yield compounds **11** (56 mg), **12** (60 mg), and **13** (120 mg).

3. Results

Compound **1**: white powder ($CHCl_3$). EI-MS and ^{1}H-NMR data of compound **1** were nearly identical to those of tetracosanic acid in the literature (Li *et al*, 2000). The characteristic fragment ions at *m/z*: 340 (M^+), 312, 298, 284, 270, 256, 242, 228, 214, 200, 129, 115, 101,87, 73, 59, 45; ^{1}H-NMR δ: 0.86 (3H, t, J = 6.4Hz, CH_3), 1.27 [m, 36H, $(CH_2)_{18}$], 1.60 (2H, t, J = 7.2 Hz), and 2.25 (2H, t, J = 7.6 Hz). The molecular weight of major fragment ions of compound **1** in EI-MS spectrum was 28 lower than that of tetracosanic acid in the literature (Li et al, 2000). Thus, compound **1** was identified as docosanoic acid.

Compound **2**: white powder ($CHCl_3$). EI-MS data of compound **2** were nearly identical to those of tricosane in the literature (Ren et al, 2000). The characteristic fragment ions at *m/z*: 338 (M^+), 85, 71, 57, 43. The molecular weight of major fragment ions of compound **2** in EI-MS spectrum was 14 higher than that of tricosane in the literature (Ren and Yang, 2000). Thus, compound **2** was identified as tetracosane.

Compound **3**: white powder ($CHCl_3$). EI-MS data of compound **3** were nearly identical to those of compound **2**. The characteristic fragment ions at *m/z*: 254 (M^+), 85, 71, 57, 43. The molecular weight of major fragment ions of compound **3** in EI-MS spectrum was 84 lower than that of compound **2**. Thus, compound **3** was identified as octadecane.

Compound **4**: white powder ($CHCl_3$). EI-MS, ^{1}H-NMR and ^{13}C-NMR data of compound **4** were nearly identical to those of tetracosanic acid in the literature (Li et al, 2000). The characteristic fragment ions at *m/z*: 396(M^+), 368, 354, 342, 129, 73, 57. ^{1}H-NMR ($CDCl_3$) δ: 0.87(3H, t, J = 6.4 Hz, CH_3), 1.28 [m, 44H, $(CH_2)_{22}$], 1.63 (2H, t, J = 7.2 Hz), 2.30 (2H, t, J = 7.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 14.1 (C-26), 22.7 (C-25), 24.7 (C-3), 31.9 (C-22), 33.7 (C-2), 178.3 (C-1). The molecular weight of major fragment ions of compound **4** in EI-MS spectrum was 28 higher than that of tetracosanic acid in the literature (Li et al, 2000). Thus, compound **4** was identified as hexacosanoic acid.

Compound **5**: white powder ($CHCl_3$). HR-ESI-MS showed a molecular ion at *m/z*: 457 ($M + H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.67 (3H, s, CH_3-18), 1.00 (3H, s, CH_3-19), 0.91 (3H, d, J = 6.4 Hz, CH_3-21), 0.84 (3H, d, J = 7.6 Hz, CH_3-26), 0.81 (3H, d, J = 7.2 Hz, CH_3-27), 0.85 (3H, t, J = 7.6 Hz, CH_3-29), 4.60 (1H, brs, H-3), 5.36 (1H, d, J = 4.4 Hz, H-6). ^{13}C-NMR ($CDCl_3$) δ: 37.0 (C-1), 31.9 (C-2), 73.7 (C-3), 42.3 (C-4), 139.7 (C-5), 122.6 (C-6), 31.9 (C-7), 31.9 (C-8), 50.0 (C-9), 37.0 (C-10), 21.0 (C-11), 40.0 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 27.8 (C-16), 56.0 (C-17), 11.8 (C-18), 19.3 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 29.2 (C-23), 45.8 (C-24), 29.7 (C-25), 19.0 (C-26), 19.8 (C-27), 23.1 (C-28), 12.0 (C-29), 173.3 (Ac), 22.7 (Ac). The spectral data were consistent with those of β-sitosterol acetate. Thus, compound **5** was identified as β-sitosterol acetate by comparison of the ^{1}H-NMR and ^{13}C-NMR data with the reported data (Wang and Zou, 2008).

Compound **6**: white powder ($CHCl_3$), mp 136−137 ^{o}C. Lieberman-Burchard reaction was positive and 5% sulfuric acid-ethanol system was purple. TLC Rf of compound **6** and β-sitosterol reference substance was the same as the β-sitosterol reference substance. Two compounds were mixed that the melting point did not drop. Thus, compound **6** was identified as β-sitosterol.

Compound **7**: white needles crystal (MeOH), mp 190−192 ^{o}C. HR-ESI-MS showed a molecular ion at *m/z*: 373 $[M + H]^+$, ^{1}H-NMR(MeOD) δ: 6.74 (1H, s, 3-H), 6.74 (1H, s, 5-H), 6.54 (1H, d, J = 16.0 Hz, 7-H), 6.32 (1H, dt, J = 16.0 Hz, 8-H), 4.20 (1H, m, 9-H), 3.84 (3H, s, 10-H), 3.84 (3H, s, 11-H), 4.85 (1H, d, J = 6.8 Hz, Glc-1), 3.40 (1H, m, Glc-2), 3.38 (1H, m, Glc-3), 3.30 (1H, m, Glc-4), 3.28 (1H, m, Glc-5), 3.67 (3H, m, Glc-6). ^{13}C-NMR (MeOD) δ: 135.3 (C-1), 154.4 (C-2), 105.5 (C-3), 135.9 (C-4), 62.6 (C-5), 154.4 (C-6), 131.3 (C-7), 130.0 (C-8), 105.5 (C-9), 57.0 (C-10), 57.0 (C-11), 100.8 (Glc-1), 73.4 (Glc-2), 76.9 (Glc-3), 70.1 (Glc-4), 76.7 (Glc-5), 61.1 (Glc-6). The spectral data were consistent with those of syringin. Thus, compound **7** was identified as syringin by comparison of ^{1}H-NMR and ^{13}C-NMR data with the reported data (Wu et al, 1999).

Compound **8**: white needles crystal (MeOH), mp 180−182 ^{o}C. HR-ESI-MS showed a molecular ion at *m/z*: 285

$[M+H]^+$, ^{1}H-NMR (CD_3OD) δ: 4.21 (2H, dd, J = 6.0, 0.8 Hz, H-2), 3.44 (1H, m, 3-H), 5.44 (1H, d, J = 6.8 Hz, H-4), 7.26 (1H, d, J = 8.0 Hz, H-5), 6.48 (1H, dd, J = 8.0, 2.0 Hz, H-6), 6.29 (1H, d, J = 2.4 Hz, H-8), 6.44 (1H, s, H-2′), 6.79 (1H, s, H-5′), 5.87 (1H, dd, J = 1.2, 0.8 Hz, -O-CH_2-O-), ^{13}C-NMR (CD_3OD) δ: 67.4 (C-2), 41.6 (C-3), 80.0 (C-4), 133.1 (C-5), 110.7 (C-6), 160.1 (C-7), 104.1 (C-8), 158.0 (C-9), 112.9 (C-10), 119.8 (C-1′), 105.9 (C-2′), 143.1 (C-3′), 149.6 (C-4′), 94.2 (C-5′), 158.0 (C-6′), 102.4 (-O-CH_2-O-). The spectral data were consistent with those of maackiain. Thus, compound **8** was identified as maackiain by comparison of ^{1}H-NMR and ^{13}C-NMR data with the reported data (Wang and Zou, 2008).

Compound **9**: yellow crystal (MeOH). HR-ESI-MS showed a molecular ion at *m/z*: 269 $[M + H]^+$. ^{1}H-NMR (DMSO-d_6) δ: 8.31 (1H,s,H-2), 7.98(1H, d, J = 8.8 Hz, H-5), 6.94 (1H, dd, J = 8.8, 2.0 Hz, H-6), 10.81 (1H, s, H-7), 6.87 (1H, d, J = 2.0 Hz, H-8), 7.50 (2H, d, J = 8.8 Hz, H-2′, 6′), 6.98 (2H, d, J = 8.8 Hz, H-3′, 5′), 3.77 (3H, s, -OCH_3). ^{13}C-NMR (DMSO-d_6) δ: 153.1 (C-2), 123.2 (C-3), 174.5 (C-4), 127.3 (C-5), 115.2 (C-6), 162.6 (C-7), 102.1 (C-8), 157.4 (C-9), 116.6 (C-10), 125.8 (C-1′), 130.1 (C-2′,6′), 113.6 (C-3′,5′), 158.9 (C-4′), 55.1 (-OCH_3). The spectral data were consistent with those of formononetin. Thus, compound **9** was identified as formononetin by comparison of ^{1}H-NMR and ^{13}C-NMR data with the reported data (Wang et al, 2008).

Compound **10**: yellow crystal (MeOH). HR-ESI-MS showed a molecular ion at *m/z*: 283 $[M + H]^+$. ^{1}H-NMR (DMSO-d_6) δ: 8.32 (1H, s, H-2), 7.97 (1H, d, J = 8.8 Hz, H-5), 6.94 (1H, dd, J = 8.8, 2.0 Hz, H-6), 10.79 (1H, s, H-7), 6.87 (1H, d, J = 2.0 Hz, H-8), 7.14 (1H, d, J = 1.6 Hz, H-2′), 6.95 (1H, d, J = 8.8 Hz, H-5′), 7.05 (1H, d, J = 1.6 Hz, H-6′), 6.03 (2H, s, -O-CH_2-O-). ^{13}C-NMR (DMSO-d_6) δ: 153.4 (C-2), 123.2 (C-3), 174.6 (C-4), 127.3 (C-5), 115.2 (C-6), 162.6 (C-7), 102.1 (C-8), 157.4 (C-9), 116.6 (C-10), 124.2 (C-1′), 109.4 (C-2′), 147.0 (C-3′), 146.9 (C-4′), 108.7 (C-5′), 122.4 (C-6′), 147.0 (-O-CH_2-O-). The spectral data were consistent with those of ψ-baptigenin. Thus, compound **10** was identified as ψ-baptigenin by comparison of ^{1}H-NMR and ^{13}C-NMR data with the reported data (Gu and Zhou, 1988).

Compound **11:** white needles crystal (MeOH). HR-ESI-MS showed a molecular ion at *m/z*: 489 $[M + H]^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.14, 0.69, 0.96, 0.78, 1.31 (each 3H, s, -CH_3), 0.92 (3H, d, J = 6.4 Hz, -CH_3), 3.60 (1H, dd, J = 6.8, 10.8 Hz, H-3), 5.29 (1H, brs, H-12). ^{13}C-NMR (DMSO-d_6) δ: 39.5 (C-1), 27.2 (C-2), 78.1 (C-3), 42.7 (C-4), 48.8 (C-5), 19.3 (C-6), 33.7 (C-7), 41.0 (C-8), 48.4 (C-9), 37.9 (C-10), 24.7 (C-11), 129.4 (C-12), 140.0 (C-13), 43.1 (C-14), 29.6 (C-15), 26.6 (C-16), 48.6 (C-17), 55.1 (C-18), 73.6 (C-19), 43.3 (C-20), 27.1 (C-21), 39.1 (C-22), 67.5 (C-23), 12.7 (C-24), 17.5 (C-25), 16.6 (C-26), 24.9 (C-27), 182.3 (C-28), 27.3 (C-29), 16.2 (C-30). The spectral data were consistent with those of rotundic acid. Thus, compound **11** was identified as rotundic acid by comparison of ^{1}H-NMR and ^{13}C-NMR data with the reported data (Ma et al, 2005).

Compound **12**: white needles crystal (MeOH). HR-ESI-MS showed a molecular ion at *m/z*: 651 $[M + H]^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.29, 1.19, 0.97, 0.92, 0.77, 0.69 (each 3H, s, -CH_3), 0.91 (3H, d, J = 6.4 Hz, -CH_3), 3.56 (1H, dd, J = 5.60, 4.80 Hz, H-3), 5.30 (1H, brs, H-12), 4.59 (1H, d, J = 7.5 Hz, Glc-1). ^{13}C-NMR (DMSO-d_6) δ: 39.4 (C-1), 26.5 (C-2), 74.1 (C-3), 39.6 (C-4), 48.8 (C-5), 17.7 (C-6), 33.5 (C-7), 41.0 (C-8), 48.4 (C-9), 37.9 (C-10), 24.7 (C-11), 129.7 (C-12), 139.6 (C-13), 42.7 (C-14), 29.6 (C-15), 27.1 (C-16), 48.8 (C-17), 54.8 (C-18), 73.7 (C-19), 43.3 (C-20), 27.0 (C-21), 41.2 (C-22), 67.6 (C-23), 12.6 (C-24), 17.6 (C-25), 17.5 (C-26), 26.4 (C-27), 178.6 (C-28), 27.2 (C-29), 16.1 (C-30), 95.8 (Glc-1), 74.1 (Glc-2), 78.6 (Glc-3), 71.1 (Glc-4), 78.3 (Glc-5), 62.4 (Glc-6). The spectral data were consistent with those of pedunculoside. Thus, compound **12** was identified as pedunculoside by comparison of ^{1}H-NMR and ^{13}C-NMR data with the reported data (Wei and Chen, 1991).

Compound **13**: white powder (MeOH), mp 290−295 °C. Lieberman-Burchard and Molish reaction was positive and 5% sulfuric acid-ethanol system was purple. TLC Rf of compound **13** and daucosterol reference substance were the same. Two compounds were mixed that the melting point did not drop. Thus, compound **13** was identified as daucosterol.

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