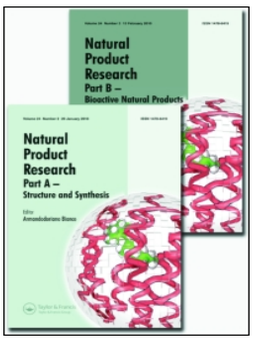

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Two new kaurane-type diterpenoids from *Wedelia chinensis* (Osbeck.) Merr

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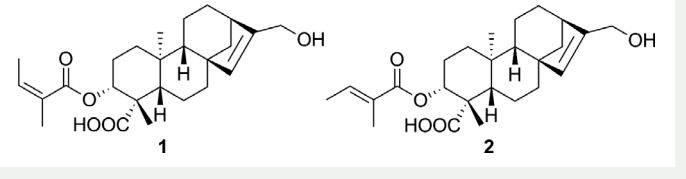
ABSTRACT

Two new kaurane-type diterpenoids, 3*α*-(angeloyloxy)-17-hydroxy-*ent*-kaur-15-en-19-oic acid (**1**) and 3*α*-(tigloyloxy)-17-hydroxy-*ent*-kaur-15-en-19-oic acid (**2**), along with 10 known compounds (**3–12**) were isolated from the whole plant of *Wedelia chinensis* (Osbeck.) Merr. Their structures were elucidated on the basis of extensive spectroscopic analyses (UV, IR, MS and NMR) and comparison with literature data. Compounds **3** and **4** showed moderate inhibitory activity against the *Staphylococcus aureus* subsp. *aureus* ATCC29213 with MIC_{50} 19.35 and 18.31 μg/mL, respectively.



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Wedelia chinensis; kaurane-type diterpenoids; antibacterial activity

1. Introduction

Wedelia chinensis (Osbeck.) Merr., a genus of the Compositae, widely distributed in the north-east, east and south provinces of China (Editorial Committee of Flora of China 1979). It is traditionally used as a medicinal herb for the treatment of diarrhoea, haemorrhoids, diphtheria, chincough, injuries and faucitis (Li et al. 2012). The extract of *W. chinensis* was reported to have extensive bioactivity, e.g. anti-inflammatory (Huang et al. 2013; Yuan et al. 2013), antimicrobial (Sureshkumar et al. 2007; Darah et al. 2013), anti-prostate cancer (Lin et al. 2007; Tsai et al. 2009). Extensive studies of the chemical components of Wedelia have led to the identification of kinds of compounds, including sesquiterpenes, Eudesmanolides, beyerene and kaurene diterpenes, triterpenes, triterpene saponins, flavonoids, etc. (Dalva et al. 1994; Valdir et al. 2004; Xing et al. 2007). The phytochemical studies of *W. chinensis* showed that it contains ent-kaurane terpenoids (Qiu et al. 2014), triterpenoid saponins (Xing et al. 2012), flavonoid glycosides, oligoglycosidic compounds, caffeic acid derivatives (Sandra

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et al. 2002), spathulenol and *β*-amyrin-acetate (Yuying et al. 1997). However, the investigation about the chemical constituents of *W. chinensis* is not sufficient compared to the other plants in the genus *Wedelia*.

In our research, two new diterpenoids, 3*α*-(angeloyloxy)-17-hydroxy-*ent*-kaur-15-en-19-oic acid (**1**) and 3*α*-(tigloyloxy)-17-hydroxy-*ent*-kaur-15-en-19-oic acid (**2**), together with 10 known compounds (**3–12**) were isolated from the dried whole plant of *W. chinensis*. The content of compound **4** in the whole plant is approximately 1% (31 g in 35 kg dried whole plant). Compounds **1–4**, **7** and **9** (Figure 1) were evaluated for their antibacterial activities against *Escherichia coli* ATCC25922, *Staphylococcus aureus* subsp. *aureus* ATCC29213, *Salmonella enterica* subsp. *Enteric* ATCC14028 and *Pseudomonas aeruginosa* ATCC27853.

2. Results and discussion

Compound **1** was isolated as white amorphous powder. Its molecular formula $C_{25}H_{36}O_5$ was determined by HR-ESI-MS at m/z 439.2462 $[M + Na]^+$ (calcd 439.2455, $[C_{30}H_{36}O_5Na]^+$), corresponding to 8 degrees of unsaturation. The IR spectrum showed absorption bonds at 3431 cm^{-1} (OH) and 1709 cm^{-1} (COOH). The ^{13}C NMR spectrum displayed 25 carbon signals, which could be assigned to a diterpenoid and an angeloyloxyl group (δ_c 167.8, 138.1, 127.9, 20.6, 15.7). The diterpenoid part was composed of two methyls, eight methylenes (one oxygenated), six methines (one oxygenated) and four quanternary carbons, which was similar to 17-hydroxy-ent-kaur-15-en-18-oic acid (**3**) (Hsieh et al. 2004). Thus, **1** had a kaurane-type diterpenoid skeleton with an angeloyloxy substituent group. The angeloyloxy group was located to C-3 by the HMBC corrections of H-3 to C-1′/C-19 and CH_3-18 to C-3. Thus, the planar structure of compound **1** was established as shown (Figure 1).

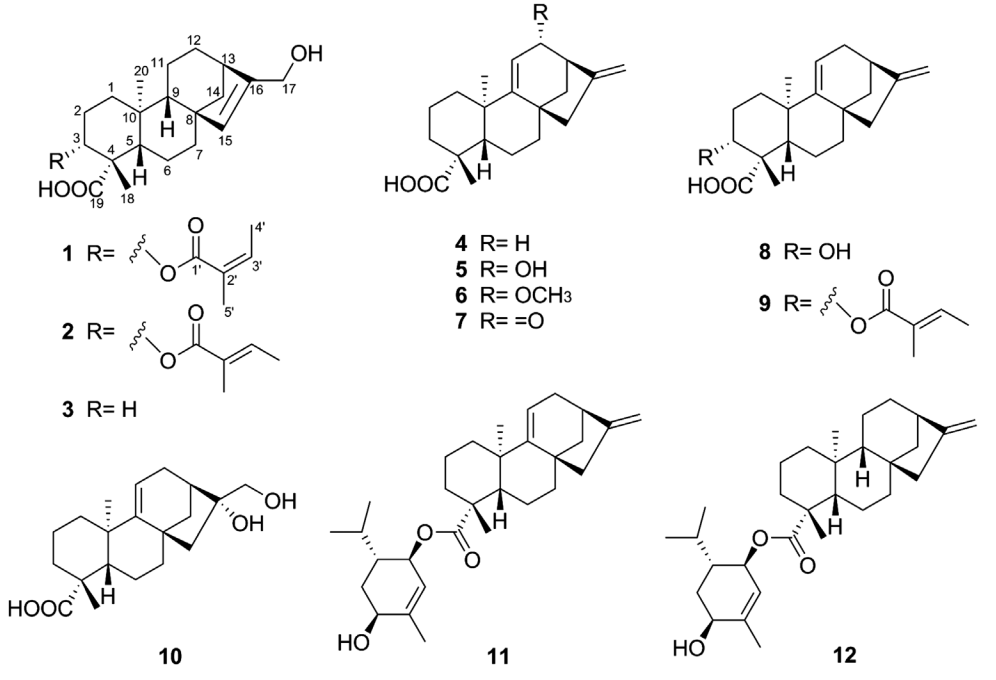

Figure 1. Chemical structures of compounds **1–12**.

The relative configuration of **1** was elucidated by its ROESY correlations. The correlations of H-3 to H-5/CH_3-18, suggested that the angeloyloxy group was *α*-orientated, while the other parts were identical with those of **3**. Thus, the structure of **1** was finally determined as 3*α*-(angeloyloxy)-17-hydroxy-*ent*-kraur-15-en-19-oic acid.

Compound **2** was isolated as white amorphous powder. Its molecular formula $C_{25}H_{36}O_5$ was determined by HR-ESI-MS at m/z 439.2465 $[M + Na]^+$ (calcd 439.2455, $[C_{30}H_{36}O_5Na]^+$), corresponding to 8 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3425 cm^{-1}) and carbonyl (1705 cm^{-1}). The NMR data of **2** had a high similarity with those of **1** indicated that both compounds shared the same basic skeleton and a similar substituent group. The chemical shifts of the two methyls (δ_c 15.7, CH_3-4′; δ_c 20.6, CH_3-5′) in ^{13}C NMR indicated the presence of a tigloyloxy group in **2** rather than an angeloyloxy group (δ_c 14.4, CH_3-4′; δ_c 12.0, CH_3-5′) in **1,** which was corresponding to the data indicated in the literature (Henriete et al. 2001). The position of tigloyloxy group was connected to C-3, which was determined by HMBC corrections of H-3 to C-1′/C-19 and CH_3-18 to C-3. The other part of **2** was identical with that of **1**. The ROESY correlations of H-3 to H-5/CH_3-18 suggested that the tigloyloxy group was *α*-orientated. Thus, the structure of **2** was finally determined as 3*α*-(tigloyloxy)-17-hydroxy-*ent*-kraur-15-en-19-oic acid.

Compounds **1–4, 7** and **9** were tested for their antimicrobial activity. Only compounds **3** and **4** showed moderate inhibitory activity against the *S. aureus* subsp. *aureus* ATCC29213 with MIC_{50} 19.35 and 18.31 μg/mL, respectively.

3. Experiment

3.1. General experimental procedures

NMR spectra were recorded on Bruker AVANCE III 400, 500 or 600 MHz spectrometer (Bruker, Karlsruhe, Germany) with tetramethylsilane as the internal standard. Optical rotation values were obtained on Jasco P-1020 automatic digital spectropolarimeter (Jasco International, Tokyo, Japan). UV spectral data were measured on Shimadzu UV-2401PC spectrophotometer (Shimadzu, Tokyo, Japan). IR was carried out on Bruker Tensor 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany), KBr pellets. ESI-MS and HR-ESI-MS were recorded on Waters Xevo TQ-S mass spectrometer (Waters Corp., Milford, MA, USA). Normal-pressure column chromatography was performed on silica gel (100–200, 300–400 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (40–70 μm, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Medium-pressure liquid chromatography was performed on MCI-gel CHP 20P gel (70–150 μm; Mitsubishi Chemical Industries Ltd., Tokyo, Japan) and Lichroprep RP-C_{18} gel (40–60 μm, Merck, Germany). Preparative high-performance liquid chromatography (HPLC) was carried out on Agilent 1200 liquid chromatography system (Agilent Technologies, Santa Clara, CA, USA) equipped with diode-array detector (DAD). The HPLC-grade acetonitrile and methanol were from Fisher Scientific (Loughborough, UK). The melting point was test by RY-1G melting point detector (Nanjing Kehang laboratory instrument Inc., Nanjing, China.)

3.2. Plant material

The whole plants of *W. chinensis* (Osbeck.) Merr. were collected at Xishuangbanna, Yunnan, China in 2014 and identified by assistant professor Yu Chen. The voucher specimen (KIB 20150508) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried whole plants of *W. chinensis* were extracted three times with methanol at room temperature. The extract solution was combined and concentrated under reduced pressure to yield crude extract. Then the crude extract was suspended in water and partitioned with petroleum ether for three times. The concentrated PE extraction (900 g) was then loaded on a silica gel column and eluted with PE/EtOAc (1:0–1:1) in a gradient mode to yield fractions A–E. Fraction A crystallised to give **4** (31 g). Fraction B was chromatographed on MCI gel and silica gel column (PE/EtOAc, 80:1–1:1) to yield three fractions B1-3, then fraction B2 crystallised to give **3 (**130 mg). Fraction C was loaded on a MCI gel column and eluted with MeOH/H_2O (50–100%) to give fractions C1-7. Fraction C3 and fraction C4 were then loaded on a RP-C_{18} column (MeOH/H_2O, 40–100%) to give Fraction C3a-f and Fraction C4a-d, respectively. Fraction C3b was loaded on Sephadex LH-20 column (MeOH) and then purified by semi-preparation HPLC to give **6** (400 mg) and **7** (14 mg). Compounds **5**, **8** and **9** (2.6 mg, 44 mg and 28 mg, respectively) were purified from fraction C3c by silica gel and semi-preparative HPLC. Fraction C4b was subjected to silica gel column to give fractions C4b1-5. Compound **10** (8 mg) was purified by HPLC (73% MeOH) from Fraction C4b2. Fraction C4b3 was a combine of **11** (10 mg) and **12** (5 mg), which were purified by HPLC (75% MeOH).

3.3.1. 3α-(angeloyloxy)-17-hydroxy-ent-kaur-15-en-19-oic acid (1)

White amorphous powder; melting point 193–195°C; $[\alpha]_D^{24}$ – 92.9 (c = 0.08, MeOH); UV (MeOH) λ_{max} 210 nm; IR (KBr) ν_{max} 3431, 2927, 2856, 1709, 1621, 1457, 1384, 1236, 1163, 1049, 989 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.97 (1H, m, H-1α), 1.06 (1H, m, H-1β), 2.40 (1H, dq, H-2α), 1.77 (1H, br.s, H-2β), 4.60 (1H, dd, J = 4.6, 12.1 Hz, H-3), 1.11 (1H, br.d, H-5), 1.62 (2H, m, H-6), 1.87 (1H, m, H-7α), 1.62 (1H, m, H-7β), 1.00 (1H, br.d, H-9), 1.64 (1H, m, H-11α), 1.54 (1H, m, H-11β), 1.50 (2H, br.s, H-12), 2.55 (1H, br.s, H-13), 2.04 (1H, br.d, H-14α), 1.41 (1H, br.q, H-14β), 5.36 (1H, s, H-15), 4.19 (2H, s, H-17), 1.29 (3H, s, H-18), 1.06 (3H, s, H-20), 6.07 (1H, q, J = 7.1, 14.0 Hz, H-3′), 1.97 (3H, d, J = 7.1 Hz, H-4′), 1.87 (3H, s, H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 38.76 (C-1), 24.09 (C-2), 78.68 (C-3), 47.93 (C-4), 56.06 (C-5), 20.46 (C-6), 38.96 (C-7), 48.56 (C-8), 47.66 (C-9), 39.49 (C-10), 18.98 (C-11), 25.39 (C-12), 40.94 (C-13), 43.65 (C-14), 135.19 (C-15), 146.28 (C-16), 61.15 (C-17), 23.90 (C-18), 180.10 (C-19), 15.30 (C-20), 167.75 (C-1′), 127.93 (C-2′), 138.12 (C-3′), 15.69 (C-4′), 20.64 (C-5′); HR-EI-MS m/z: 439.2462 $[M + Na]^+$ for $C_{25}H_{36}O_5$.

3.3.2. 3α-(tigloyloxy)-17-hydroxy-ent-kaur-15-en-19-oic acid (2)

White amorphous powder; melting point 221–223°C; $[\alpha]_D^{24}$ – 41.1 (c = 0.08, MeOH); UV (MeOH) λmax 209 nm; IR (KBr) ν_{max} 3425, 2928, 2856, 1705, 1608, 1384, 1272, 1130, 1081, 1023 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 1.97 (1H, m, H-1α), 1.05 (1H, m, H-1β), 2.35 (1H, br.q, H-2α), 1.72 (1H, m, H-2β), 4.56 (1H, dd, J = 4.6, 12.1 Hz, H-3), 1.11 (1H, br.d, H-5), 1.86 (2H, br.d, H-6), 1.57 (2H, m, H-7), 1.00 (1H, br.d, H-9), 1.54 (2H, m, H-11), 1.49 (2H, m, H-12), 2.54 (1H, br.s, H-13), 2.04 (1H, d, H-14α), 1.40 (1H, br.q, H-14β), 5.35 (1H, s, H-15), 4.18 (2H, s, H-17), 1.26 (3H, s, H-18), 1.05 (3H, s, H-20), 6.86 (1H, q, J = 7.1, 14.0 Hz, H-3′), 1.77 (3H, d, J = 7.1 Hz, H-4′), 1.81 (3H, s, H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 38.75 (C-1), 23.98 (C-2), 78.75 (C-3), 48.01 (C-4), 56.04 (C-5), 20.47 (C-6), 38.95 (C-7), 48.56 (C-8), 47.61 (C-9), 39.49 (C-10), 18.99 (C-11), 25.38 (C-12), 40.94 (C-13), 43.59 (C-14), 135.19 (C-15), 146.29 (C-16), 61.18 (C-17), 23.86 (C-18), 179.80 (C-19), 15.29 (C-20), 167.73 (C-1′), 128.73 (C-2′), 137.43 (C-3′), 14.42 (C-4′), 11.99 (C-5′); HR-EI-MS m/z: 439.2465 $[M + Na]^+$ for $C_{25}H_{36}O_5$.

3.4. Antibacterial activity

Escherichia coli ATCC25922, *S. aureus* subsp. *aureus* ATCC29213, *S. enterica* subsp. *Enteric* ATCC14028 and *P. aeruginosa* ATCC27853 were purchased from China general microbiology preservation management centre. The reagents that had used in this assay were Ceftazidime (Yuanye Biotechnology Corp., Shanghai), Penicillin G sodium (Biosharp Corp.), DMSO (Sigma Corp.), broth (Huankai Biotechnology Corp., Guangdong) and agar powder (Scientific Research Special Corp.). The samples were diluted into 96-well culture plate and added the bacteria inoculum (5×10^5 CFU/mL per well), then the mixture were incubated at 37°C for 24 h. The optical density was obtained at 655 nm. Blank and positive control (Ceftazidime, Penicillin G sodium) were also set during the test.

4. Conclusion

Two new kaurane diterpenoids, 3*α*-(angeloyloxy)-17-hydroxy-ent-kaur-15-en-19-oic acid (**1**) and 3*α*-(tigloyloxy)-17-hydroxy-ent-kaur-15-en-19-oic acid (**2**), along with 10 known compounds (**3–12**) were isolated from the whole plant of *W. chinensis*. There are abundant diterpenoids in *W. chinensis* and the content of compound **4** in the whole plant is approximately 1% (31 g in 35 kg dried whole plant). In the antibacterial assay, Compounds **3** and **4** showed moderate inhibitory activities against the *S. aureus* with MIC_{50} 19.35 and 18.31 μg/mL, respectively.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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