Four new ent-kaurene diterpene glucosides from Mikania micrantha

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Abstract

Phytochemical study on the aerial parts of Mikania micrantha led to the isolation of four new ent-kaurene diterpene glucosides, β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (1), β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (2), β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (3), β-D-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (4), along with a known one, β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-ent-16-kauren-19-oate (5). Their structures were elucidated on the basis of extensive spectroscopic analysis. Compounds 1–4 are a group of C-9 hydroxylated ent-kaurene diterpene glucosides which is relatively rare in nature. These compounds selectively showed in vitro antibacterial activity against four assayed Gram-(+) and three Gram-(-) bacteria. In addition, the in vitro growth inhibitory activity of these compounds against human cancer cell lines Hela, A549, HepG-2 and MCF-7, were also tested.

Full Text

Preamble

Four New ent-Kaurene Diterpene Glucosides from *Mikania micrantha*

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ABSTRACT

Phytochemical investigation of the aerial parts of *Mikania micrantha* led to the isolation of four new ent-kaurene diterpene glucosides: β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (1), β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (2), β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (3), and β-D-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (4), along with a known compound, β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-ent-16-kauren-19-oate (5). Their structures were elucidated through extensive spectroscopic analysis. Compounds 1–4 represent a relatively rare group of C-9 hydroxylated ent-kaurene diterpene glucosides found in nature. These compounds exhibited selective in vitro antibacterial activity against four Gram-positive and three Gram-negative bacterial strains. Additionally, their in vitro growth inhibitory activity against human cancer cell lines HeLa, A549, HepG-2, and MCF-7 was evaluated.

Keywords: *Mikania micrantha*; ent-Kaurane; Diterpene glucosides; Antibacterial activity; Cytotoxicity

1. Introduction

Mikania micrantha H. B. K. (Asteraceae) is a perennial creeping vine native to Central and South America. Outside its native range, it exhibits rapid growth and has become an invasive exotic species in Southeast and South Asia, causing substantial economic losses in agricultural and forestry production in affected regions (Feng et al., 2002; Zhang et al., 2004). In Mexico, *M. micrantha* has long been used as a traditional folk remedy for treating skin diseases, snake bites, and scorpion stings (Aguilar, 1994). Previous phytochemical studies have identified structurally diverse compounds from this plant, including terpenoids, steroids, flavonoids, and phenolic compounds, several of which display significant bioactivities (Boeker et al., 1987; But et al., 2009; Cuenca et al., 1988; Herz et

al., 1975; Huang et al., 2004; Nicollier and Thompson, 1981; Ríos et al., 2014; Wei et al., 2004; Xu et al., 2013a, 2013b). As part of our ongoing research to discover novel bioactive natural products from invasive plants in China (Luo et al., 2015; Ren et al., 2015; Yan et al., 2010; Zhang et al., 2014; Zhou et al., 2013), we conducted a phytochemical investigation of the aerial parts of *M. micrantha*, which yielded four new C9-hydroxylated ent-kaurene diterpene glucosides (1–4) along with one known diterpene glucoside (5). This paper reports the isolation, structural elucidation, and in vitro antibacterial and cytotoxic evaluation of these compounds.

2. Results and Discussion

Compound 1 was isolated as a white amorphous powder with a molecular formula of $C_{31}H_{48}O_{11}$, as determined by HR-ESI-MS data showing m/z 631.2895 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{11}Cl^-$, 631.2891), indicating eight degrees of unsaturation. The ^{1}H NMR spectrum displayed signals for four tertiary methyl groups at δH 1.12 (3H, s, Me-20), 1.24 (3H, s, Me-18), 1.31 (3H, s, Me-4), and 1.31 (3H, s, Me-5), an oxymethine at δH 6.02 (1H, s, H-15), and an exomethylene at δH 5.09 and 5.17 (1H each, s, H-17). The ^{13}C NMR spectrum , combined with HSQC analysis, revealed thirty-one carbons, including four methyls [δC 18.2 (C-20), 29.1 (C-18), 29.3 (C-4), and 29.6 (C-5)], eleven methylenes, eight methines, and eight quaternary carbons [including two oxygenated quaternary carbons at δC 70.4 (C-3) and 77.8 (C-9)], two carboxyl groups [δC 178.5 (C-19) and 172.9 (C-1)], and an exocyclic olefinic group [δC 110.6 (C-17) and 157.5 (C-16)]. The presence of a β-D-glucopyranosyl moiety was indicated by signals at δH 5.44 (1H, d, J = 8.0 Hz, H-1) and δC 62.4 (C-6), 71.1 (C-4), 74.0 (C-2), 78.7 (C-3), 78.7 (C-5), and 95.6 (C-1). Careful analysis of the NMR data revealed that compound 1 closely resembled β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-ent-16-kauren-19-oate (Xu et al., 2013a), a known ent-kaurene diterpene glucoside (5) also isolated in this study. The key difference was that the methine group signals at C-9 [δH 1.19 (1H, m, H-9); δC 54.5 (C-9)] in 5 were replaced by a hydroxylated quaternary carbon signal [δC 77.8 (C-9)] in 1. These observations led us to establish the structure of 1 as shown in [Figure 1: see original paper]. This assignment was consistent with the molecular formula and supported by HMBC correlations from δH 1.12 (H-20), 1.57 (H-12), and 6.02 (H-15) to δC 77.8 (C-9). Additionally, the HMBC correlation from δH 5.44 (H-1) to δC 178.5 (C-19) [Figure 2: see original paper] confirmed the location of the glucopyranosyl moiety at C-19. HMBC correlations from δH 1.31 (6H, s, H-4 and H-5) to δC 48.9 (C-2) and from δH 2.48 and 2.51 (1H each, d, J = 14.1 Hz, H-2) to δC 172.9 (C-1) [Figure 2: see original paper] verified the presence of a 3-hydroxy-3-methylbutanoyloxy moiety (Xu et al., 2013a). Furthermore, HMBC correlations from δH 6.02 (H-15) to C-9, C-13, C-14, and C-1 [Figure 2: see original paper] supported the attachment of the butanoyloxy moiety at C-15. The observed NOE correlation between H-15 and Me-18 indicated the β-orientation of H-15. Thus, compound 1 was identified as β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-

hydroxy-ent-16-kauren-19-oate.

Compound 2 was also obtained as a white amorphous powder. Its molecular formula $C_{31}H_{48}O_{10}$ was established from HR-ESI-MS data showing m/z 615.2936 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941). The NMR data of 2 closely matched those of 1 [TABLE:1 and TABLE:2], suggesting it was also an ent-kaurene diterpene glucoside. Detailed comparison revealed that the signal for the hydroxylated quaternary carbon C-3 in 1 was absent in 2, with additional signals for a methine group [δH 2.09 (1H, m, H-3), δC 27.0 (C-3)] appearing instead. This indicated that 2 shared the same basic structure as 1, differing only by replacement of the hydroxylated C-3 with a methine group. This conclusion was supported by the molecular formula, the altered chemical shifts and coupling constants of CH_2-2 , Me-4 , and Me-5 compared to 1 (see TABLE:1 and TABLE:2), and HMBC correlations from δH 0.98 (6H, d, J = 6.6 Hz, H-4 and H-5) to δC 44.8 (C-2) and from δH 2.19 (2H, dd, J = 7.1, 1.6 Hz, H-2) to δC 174.6 (C-1) [Figure 2: see original paper]. Therefore, compound 2 was elucidated as β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate.

Compound 3 had the same molecular formula as 2, $C_{31}H_{48}O_{10}$, based on HR-ESI-MS analysis showing m/z 615.2942 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941). Detailed comparison revealed that the ^{1}H and ^{13}C NMR data [TABLE:1 and TABLE:2] of 3 were nearly identical to those of 2, except that the signals for the 3-methylbutanoyloxy moiety at C-15 in 2 were replaced by signals for a 2-methylbutanoyloxy moiety in 3. This supported the assignment of 3 as a close analog of 2, differing only by the shift of one methyl group from C-3 in 2 to C-2 in 3. This deduction was fully consistent with the observed changes in 1D NMR data from C-1 through C-5 [TABLE:1 and TABLE:2] and was further corroborated by HMBC correlations from δH 1.14 (3H, d, J = 7.0 Hz, H-5) to δC 178.1 (C-1) and from δH 0.93 (3H, t, J = 7.4 Hz, H-4) to δC 43.1 (C-2) [Figure 2: see original paper]. Consequently, compound 3 was established as β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate.

The HR-ESI-MS spectrum of compound 4 established its molecular formula as $C_{31}H_{46}O_{10}$, with m/z 613.2791 $[M + Cl]^-$ (calcd for $C_{31}H_{46}O_{10}Cl^-$, 613.2785). Comparison of its ^{1}H and ^{13}C NMR data with those of 2 revealed similar patterns, except that the methylene group at C-2 and methine group at C-3 in 2 were absent in 4 [TABLE:1 and TABLE:2]. Instead, signals for a trisubstituted double bond (-CH(2)=C(3)-) appeared as a singlet proton resonance at δH 5.65 and two carbon signals at δC 117.3 and 157.7. This supported the assignment of 4 as a close analog of 2, differing only by the presence of a double bond between C-2 and C-3 . This assignment was consistent with the molecular formula of 4 and supported by HMBC correlations from δH 1.91 (3H, d, J = 1.0 Hz, H-4) and 2.16 (3H, d, J = 1.0 Hz, H-5) to δC 117.3 (C-2) and from δH 5.65 (1H, br s, H-2) to δC 168.1 (C-1) [Figure 2: see original paper]. Therefore, compound 4 was identified as β-D-glucopyranosyl-15α-(3-methyl-2-

butenoyloxy)-9β-hydroxy-ent-16-kauren-19-oate.

The known compound 5 was identified as β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-ent-16-kauren-19-oate by comparison of its spectral data (^{1}H and ^{13}C NMR and MS) with literature values (Xu et al., 2013a). Compounds 1–4 constitute a relatively rare group of C-9 hydroxylated ent-kaurene diterpene glucosides. A literature survey revealed that only a few C-9 hydroxylated ent-kaurene diterpene glucosides have been reported to date (Ohtani et al., 1992; Richter et al., 1977; Shimizu et al., 1990; Takahashi et al., 2004; Tanaka et al., 1981; Tellez et al., 2004; Torrenegra et al., 1999).

The in vitro antibacterial activity of compounds 1–5 was evaluated against four Gram-positive bacteria (*Bacillus subtilis*, *Curtobacterium flaccumfaciens*, *Bacillus cereus*, and *Staphylococcus aureus*) and three Gram-negative bacteria (*Salmonella typhimurium*, *Pseudomonas solanacearum*, and *Escherichia coli*) using a microdilution titer assay as previously described (Wang et al., 2014). Compounds 1–5 showed weak antibacterial activity against all tested bacterial strains.

Compounds 1–5 were also evaluated for in vitro growth inhibitory activity against four human cancer cell lines (HeLa, A549, HepG-2, and MCF-7) using the MTT method (Fu et al., 2014). As shown in , compounds 1–5 exhibited moderate and selective cytotoxicity against the four tumor cell lines, with IC_{50} values ranging from 25.86 to 66.26 M, though their activities were weaker than the reference compound adriamycin (IC_{50} 1.87–3.06 M).

3.1. General Experimental Procedures

High-resolution ESI-MS was performed on a Bruker Bio TOF IIIQ spectrometer (Bruker Daltonics, USA). NMR spectra were recorded on a Bruker DRX-500 NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). Optical rotations were measured on a Perkin-Elmer Model 341 polarimeter (Perkin-Elmer, Inc., Waltham, MA). UV spectra were acquired on a Perkin-Elmer Lambda 650 UV-vis spectrometer (Perkin-Elmer, Inc., Waltham, MA). Medium-pressure liquid chromatography (MPLC) was conducted on a CXTH P3000 instrument (Beijing Chuang Xin Tong Heng Science and Technology Co., Ltd., Beijing, China) equipped with a UV 3000 UV-vis detector and a C-18 column (50 m, 50$\times500mm).PreparativeHPLCwasperformedusingaShimadzuLC-6ADpumpandaShimadzuRID-10ArefractiveindexdetectorwithaShim-packPRC-ODSC-18column(5\mu m,20\times250mm).Columnchromatography(CC)wascarriedoutusingsilicagel($20(PharmaciaFineChemicalCo.,Ltd.,Uppsala,Sweden).Analytical-gradeethylacetate,chloroform,methanol,petroleumether(b.p.60–90°C),andn-butanolwerepurchasedfromTianjinFuyuFineChemicalIndustryCo.(Tianjin,China).Thin-layerchromatography(TLC)wasperformedonprecoatedsilicagelplates(HSGF254,YantaiJiangyouSilicaGel$ in ethanol followed by heating.

3.2. Plant Material

The aerial parts of *M. micrantha* were collected in Guangzhou, China, in June 2012 and identified by Prof. Hong-Feng Chen at the South China Botanical Garden, Chinese Academy of Sciences (CAS). A voucher specimen (No. 20120615) was deposited at the Laboratory of Bioorganic Chemistry, South China Botanical Garden, Chinese Academy of Sciences.

3.3. Extraction and Isolation

The air-dried aerial parts of *Mikania micrantha* (25 kg) were powdered and extracted three times with 95% EtOH at room temperature for three days each. After concentration under vacuum, the EtOH extract was suspended in water and sequentially partitioned three times each with petroleum ether, EtOAc, and n-butanol. The EtOAc-soluble fraction (300 g) was subjected to silica gel column chromatography, eluted with $CHCl_3$/MeOH (from 100:0 to 0:100, v/v, each 21 L) to afford fractions E1–E12. Fraction E9 (18.6 g), eluted with $CHCl_3$/MeOH (90:10), was separated by MPLC using a MeOH/H_2O gradient (10:90–100:0) at 10 mL/min to yield subfractions E9-1–E9-30. Subfraction E9-17, eluted with MeOH/H_2O (60:40), was applied to Sephadex LH-20 CC with $CHCl_3$/MeOH (1:4, v/v) to give fractions E9-17-1–E9-17-8. Fraction E9-17-1 was further purified by preparative HPLC on a Shim-pack PRC-ODS C-18 column (5 m, 20$×250mm)using22{2}O(70 : 30), wassubjectedtoSephadexLH − 20CCwithCHCl{3}/MeOH(1 : 4, v/v)togivefractionsE9 − 24 − 1–E9 − 24 − 4.FractionE9 − 24 − 1wasfurtherpurifiedbypreparativeHPLConaShim − packPRC − ODSC − 18column(5μm, 20×250mm)with32_{2}$O (85:15), was separated by Sephadex LH-20 CC eluted with methanol and then purified by HPLC using 65% methanol in water (v/v) at 10 mL/min to afford compound 5 (40 mg, tR = 81 min).

3.4. Spectral Data of Compounds 1–4

β-D-glucopyranosyl-15α-(3-hydroxy-3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (1): White amorphous powder; $[\alpha]D^{20}$ -46.3 (c 0.87, CH_3OH); UV (CH_3OH) λmax nm (log) 220 (2.83); HR-ESI-MS (neg.), m/z 631.2895 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{11}Cl^-$, 631.2891); ^1H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see and .

β-D-glucopyranosyl-15α-(3-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (2): White amorphous powder; $[\alpha]D^{20}$ -11.7 (c 0.90, CH_3OH); UV (CH_3OH) λmax nm (log) 220 (3.28); HR-ESI-MS (neg.), m/z 615.2936 $[M + Cl]^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941); ^1H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see and .

β-D-glucopyranosyl-15α-(2-methylbutanoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (3): White amorphous powder; $[\alpha]D^{20}$ -38.5 (c 0.69, CH_3OH); UV (CH_3OH) λmax nm (log) 220 (3.32); HR-ESI-MS (neg.), m/z 615.2942

[M + Cl]$^-$ (calcd for $C_{31}H_{48}O_{10}Cl^-$, 615.2941); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see and .

β-D-glucopyranosyl-15α-(3-methyl-2-butenoyloxy)-9β-hydroxy-ent-16-kauren-19-oate (4): White amorphous powder; [α]D^{20} -42.0 (c 0.19, CH_3OH); UV (CH_3OH) λmax nm (log) 220 (4.03); HR-ESI-MS (neg.), m/z 613.2791 [M + Cl]$^-$ (calcd for $C_{31}H_{46}O_{10}Cl^-$, 613.2785); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR in CD_3OD, see and .

3.5. Antibacterial Activity Assay

Seven microorganisms were used in the bioassay: four Gram-positive bacteria (*Bacillus subtilis*, *Curtobacterium flaccumfaciens*, *Bacillus cereus*, and *Staphylococcus aureus*) and three Gram-negative species (*Salmonella typhimurium*, *Pseudomonas solanacearum*, and *Escherichia coli*), all obtained from the Microbial Culture Collection Center of Guangdong Institute of Microbiology (Guangzhou, China). Bacterial cultures were maintained in 20% glycerol-water medium at -80°C. Resazurin and kanamycin sulfate were purchased from Sigma Chemical Co. (Sigma-Aldrich, St. Louis, MO, USA). The assay was performed according to previously described procedures (Wang et al., 2014).

3.6. Cytotoxicity Assay

Four human cancer cell lines (HeLa, A549, HepG-2, and MCF-7) were obtained from the Cell Bank of Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and adriamycin (ADM) were purchased from Sigma Chemical Co. (Sigma-Aldrich, St. Louis, MO, USA). The assay was conducted following previously described procedures (Fu et al., 2014).

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Figure Captions

Fig. 1. Chemical structures of compounds 1–5

Fig. 2. Selected HMBC correlations (arrows) of compounds 1–4

Tables

^{1}H NMR (500 MHz) assignments [δ (ppm), J in Hz] of compounds 1–4 in CD_3OD

^{13}C NMR (125 MHz) assignments [δ (ppm)] of compounds 1–4 in CD_3OD

Cytotoxicity of compounds 1–5 (IC_{50}, M)

Note: Figure translations are in progress. See original paper for figures.