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Two new 3-hexenol glycosides from the calyces of *Physalis alkekengi* var. *franchetii*

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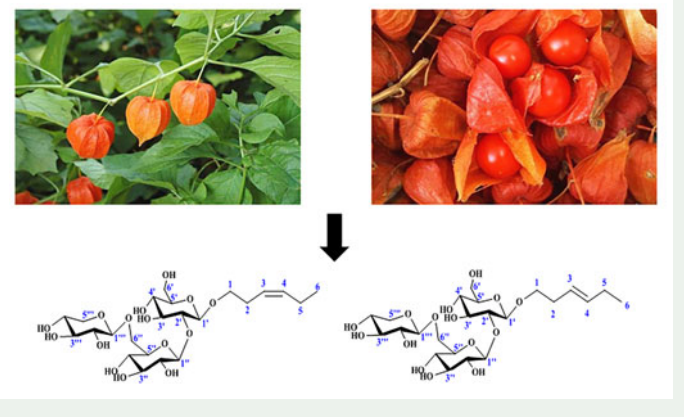
ABSTRACT

Two new hexenol glycosides, (*Z*)-hex-3-en-1-ol O-*β*-D-xylcopyranosyl-(1-6)-*β* -D-glucopyranosyl-(1-2)-*β*-D-glucopyranoside (**1**) and (*E*)-hex-3-en-1-ol O-*β*-D-xylcopyranosyl-(1-6)-*β*-D-glucopyranosyl-(1-2)-*β*-D-glucopyranoside (2), were isolated from the 50% ethanol elution of macroporous resin of *Physalis alkekengi* var. *franchetii*. Their structures were established by detailed spectroscopic analysis, including extensive 2D-NMR data. This is the first time to report the (*Z*) and (*E*) 3-hexenol glycosides from *Physalis alkekengi* var. *franchetii*.



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1. Introduction

Physalis alkekengi L. var. *franchetii* (Solanaceae) (Chinese name: Jin denglong) are used as a traditional Chinese herbal medicine for the treatment of sore throat, cough, eczema, hepatitis, urinary problems, and tumors (Shu et al. 2016). For centuries, the fruits, roots, calyces and whole plants have been used in traditional Chinese

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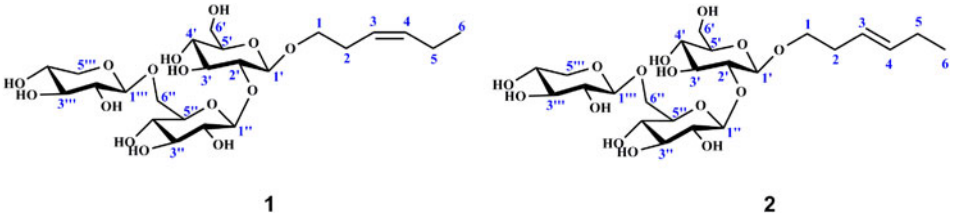
prescriptions, particularly its calyces having been considered to be one of the most important medicines, since compounds isolated from it display a wide spectrum of biological activities such as anti-trypanosomiasis, immunomodulatory, antitumor, antimicrobial, cytotoxic, molluscicidal and anti-inflammatory effects (Ge et al. 2009; Kang et al. 2011; Li et al. 2014; Yang et al. 2015; Li et al. 2018; Wang et al. 2018).

In some previous studies, flavonoids and physalins have always been considered as the main active components of *P. alkekengi* L. var. *Franchetii* (Li et al. 2012; Ooi et al. 2013). However, in the recent research, we isolated the two new 3-hexenol glycosides from the 50% ethanol elution of macroporous resin of 70% ethanol extract of the calyces of *P. alkekengi* L. var. *Franchetii.* And we tested the antimicrobial activities of the two new compounds. Unfortunately, two new compounds showed no antimicrobial activities. Interestingly, by reviewing the literature, this type of compounds was found to have anti-insect activity (Sugimoto et al. 2014). Therefore, we will test the anti-insect activity of these compounds in the future.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder. The molecular formula of **1** was determined as $C_{23}H_{40}O_{15}$ by the HR-ESI-MS at m/z 579.2279 $[M+Na]^+$, (calcd 579.2265), as well as from its NMR spectroscopic data. Two olefinic protons emerged in the high frequency of the ^{1}H-NMR spectrum at δ_H 5.35 (1H, dt, $J = 6.4$, 12.0 Hz) and 5.40 (1H, dt, $J = 6.4$, 12.0 Hz), correlated with δ_C 132.9 and 125.5, in the HSQC spectrum. Considering the two high frequency tertiary-carbon signals (δ_C 132.9 and 125.5) found in ^{13}C-NMR and DEPT spectra, it is believed that the molecule contained one –CH = CH– fragmen with (*Z*)-configuration, as indicated by the coupling constant (J_{H3-H4} = 12.0 Hz). The ^{1}H-NMR spectrum revealed signals of oxymethylene protons at δ_H 3.67 (1H, overlapped) and 3.47 (1H, overlapped), correlated with δ_c 68.4 in the HSQC spectrum, revealed the presence of a $-CH_2-O-$ fragment. Two methylene protons at δ_H 2.26 (2H, m), 2.01 (2H, m), and a methyl proton at δ_H 0.92 (3H, t, $J = 7.6$ Hz), which were correlated with δ_C 68.4, 27.6, 20.3, and 14.4 in the HSQC spectrum, suggesting that the aglycone was (*Z*)-3-hexenol (Zheng et al. 2010). Acid hydrolysis of **1** yielded D-glucose and D-xylose in a ratio of 2:1, and their configurations of the monosaccharides were determined by GC analysis of the trimethylsilyl ethers derivatives (Suzuki et al. 1993). Three anomeric protons at δ_H 4.36 (1H, d, $J = 8.0$ Hz), δ_H 4.30 (1H, d, $J = 7.6$ Hz), and δ_H 4.19 (1H, d, $J = 6.0$ Hz) were observed in the ^{1}H-NMR spectrum, correlated with δ_C 104.3, 103.6, and 101.3, respectively, in the HSQC spectrum. On the basis of ^{13}C-NMR, DEPT, HSQC, HMBC and chemical reactions, one D-glucopyranose (δ_C 101.3, 82.5, 76.0, 70.7, 77.2, 61.0), one D-glucopyranose (δ_C 104.3, 69.9, 75.1, 67.5, 76.2, 68.1) and one D-xylcopyranose (δ_C 103.6, 72.7, 75.5, 69.9, 65.1) were identified. The anomeric centers of the two glucose units and one xylose were identified as β-configuration based on large $^3J_{H\text{-}1,\ H\text{-}2}$ values (8.0, 7.6, and 6.0 Hz). Therefore, compound **1** was considered to be composed of two β-D-glucopyranose, one β-D-xylcopyranose and an aglycone with (*Z*)-3-hexenol (Figure 1). The NMR spectra data was shown in Supplementary material Table S1.



1 2

Figure 1. The structure of compound **1** and **2**.

In the HMBC spectrum, key correlation peaks were observed between the proton signal at δ_H 4.30 (H-1′) and the carbon resonance signal at δ_C 68.4 (C-1), δ_H 4.36 (H-1″) and δ_C 82.5 (C-2′), δ_H 4.19 (H-1‴) and δ_C 68.1 (C-6″). Therefore, the sequence and the attachment of the saccharide chain could be set down (Supplementary material Figure S2).

Therefore, the structure of compound **1** was determined as (*Z*)-hex-3-en-1-ol O-*β*-D-xylcopyranosyl-(1-6)-*β*-D-glucopyranosyl-(1-2)-*β*-D-glucopyranoside, and the NMR spectra data are shown in Supplementary material Table S1.

Compound **2** was obtained as a white amorphous power. The HR-ESI-MS spectrum of **2** (*m/z* 579.2273 $[M + Na]^+$, calcd for $C_{23}H_{40}O_{15}Na$, 579.2265) supported a molecular formula of $C_{23}H_{40}O_{15}$.

The sugar moieties of **2** were determined to be D-glucose and D-xylose in a ratio of 2:1, as determined by acid hydrolysis described in the part of '3.5. Acid hydrolysis', and their configurations of the component monosaccharides were determined by GC analysis. The ^{1}H-NMR spectrum (Supplementary material Table S1) showed a methyl proton at δ_H 0.85 (3H, t, $J = 7.6$ Hz), two methylene protons at δ_H 2.00 (2H, m) and 2.13 (2H, m), an oxymethylene protons at δ_H 3.63 (1H, overlapped) and 3.47 (1H, overlapped), two olefin proton signals at δ_H 5.16 (1H, dt, $J = 16.0$, 6.0 Hz) and 5.40 (1H, dt, $J = 16.0$, 6.0 Hz), in addition to the carbon signal at δ_C 13.9, 25.7, 31.1, 68.5, 127.0, and 135.0, revealed the aglycone of **2** was also 3-hexenol; signals for three anomeric protons at δ_H 4.21 (1H, d, $J = 6.0$ Hz), 4.32 (1H, d, $J = 7.6$ Hz), and 4.38 (1H, d, $J = 8.0$ Hz). On the basis of ^{13}C-NMR, DEPT, HSQC, HMBC correlations and chemical reactions, two *β*-D-glucopyranose (δ_C 101.1, 82.0, 75.8, 69.5, 76.9, 63.0 and δ_C 104.0, 70.4, 76.0, 67.4, 75.2, 67.8) and one *β*-*D*-xylcopyranose (δ_C 103.3, 71.9, 75.7, 69.2, 64.7) were identified. In the HMBC spectrum, key correlation peaks were observed between the proton signal at δ_H 4.32 (H-1′) and the carbon resonance signal at δ_C 68.5 (C-1), δ_H 4.38 (H-1″), and δ_C 82.0 (C-2′), and between the proton signal δ_H 4.21 (H-1‴) and δ_C 67.8 (C-6″). All in all, compound **2** could be determined as a 3-hexenol glycoside, as well. Analysis of the ^{1}H- and ^{13}C-NMR spectra established that the NMR spectra of compound **2** were very similar to **1**, except for a few signals due to a different double bond configuration. Compared with **1**, the *Z*-geometry of the Δ^3-double bond of **1** changed into *E*-geometry of **2**, which could be deduced from the large coupling constant observed for H-3 and H-4 ($J = 16.0$ Hz).

Therefore, the structure of **2** was determined as (*E*)-hex-3-en-1-ol O-*β*-D-xylcopyranosyl-(1-6)-*β*-D-glucopyranosyl-(1-2)-*β*-D-glucopyranoside (Figure 1), and the NMR spectra data are shown in Supplementary material Table S1.

In this activity study, the antibacterial activity of compound **1** and **2** was quantitatively assessed by determining the MIC and MBC against seven bacterial strains, including five Gram-positive bacteria (including *Staphylococcus aureus* ATCC 26112, *Staphylococcus epidermidis* ATCC 27342, *Staphylococcus saprophyticus* ATCC 24582, *Enterococcus faecium* ATCC 35667, and *Streptococcus pneumoniae* NCTC 7465) and two Gram-negative bacteria (including *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 87394). Unfortunately, we found that two compounds did not possessed antibacterial potential against each of the tested strains.

3. Experimental

3.1. General

Open column chromatography (Metrailler-Ruchonnet et al. 2010) was carried out using silica gel (200-300 mesh, Qingdao Marine Chemical Co. (Qingdao, China). TLC employed precoated silica gel plates (5–7 μm, Qingdao Marine). Preparative HPLC was carried out on a Waters 600 instrument equipped with a Waters UV-2487 detector. A Waters Sunfire prep ODS (19 $\times$ 250 mm i.d.) column was used for this purpose. The HR-ESI-MS was determined on IonSpec Ultima 7.0 T FTICR MS. The ^{1}H-, ^{13}C- and 2D-NMR (HSQC and HMBC) spectra were recorded on a Bruker AMX-400 spectrometer using standard pulse sequences. Chemical shifts are reported in ppm (δ), and scalar coupling is reported in Hz. Beef extract, peptone, and agar powder were purchased from AoboxingBio-techCo. (Beijing, China). Seven bacterial strains, including five Gram-positive bacteria, *Staphylococcus aureus* (ATCC 26112), *Staphylococcus epidermidis* (ATCC 27342), *Staphylococcus saprophyticus* (ATCC 24582), and *Enterococcus faecium* (ATCC 35667), *Streptococcus pneumoniae* (NCTC 7465), and two Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 87394) were obtained from Beijing ZK Kangtai Biological Co. (Beijing, China). Other reagents were purchased from various commercial sources.

3.2. Plant material

The calyces of *Physalis alkekengi* L. var. *franchetii*. were collected from Shangzhi, Heilongjiang province in February 2017 and identified by Prof. Wei Ma (Heilongjiang University of Traditional Chinese Medicine, Heilongjiang, China). The voucher specimen was authenticated and is deposited in the Heilongjiang University of Chinese Medicine (No.2017-02-1).

3.3. Extraction and isolation

The air-dried calyces of *Physalis alkekengi* L. var. *franchetii* (10 kg) were extracted two times with 70% EtOH for 2 h at 60 °C. The solvent was evaporated to dryness, and the dry residue (2.1 kg) was subjected to AB-8 macroporous adsorptive resin, eluted with water, 50% EtOH, and 95% EtOH respectively to yield three fractions (Fractions 1 $\sim$ 3) (Shu et al. 2016). Fraction 2 (250 g) was separated by column chromatography on silica gel eluting with CH_2Cl_2-MeOH (10:1, 5:1, 1:1, and MeOH) to give nine fractions

(Fr.$_1$∼Fr.$_9$). Fr.$_9$ was separated by column chromatography on silica gel eluting with CH_2Cl_2-MeOH (8:1, 5:1, and MeOH) to yield three fractions (Fr.$_{9\text{-}1}$∼Fr.$_{9\text{-}3}$). Fr.$_{9\text{-}2}$ was separated by column chromatography on silica gel eluting with CH_2Cl_2-MeOH (9:1 and MeOH) to yield give twelve fractions (Fr.$_{9\text{-}2\text{-}1}$∼Fr.$_{9\text{-}2\text{-}12}$). Fr.$_{9\text{-}2\text{-}4}$ was further purified by HPLC [MeOH-H_2O (30:70 v/v)] to give **1** (101 mg, 0.0404%) and **2** (134 mg, 0.0536%). The thin-layer chromatography of compound **1** and **2** showed a grey-brown spot (Rf 0.58 ∼ 0.60) with H_2SO_4/C_2H_5OH (1:9, v/v) as chromogenic reagent and the upper layer of n-butyl alcohol/acetic acid/H_2O (4:1:5, v/v/v) as developing solvent.

3.4. New compounds information

Compound **1**: $[\alpha]_D^{24}$: −56.7 °C (c = 1.00, MeOH); HR-ESI-MS: *m/z* 579.2279 $[M + Na]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR data (DMSO-d_6, 100 MHz), shown in Supplementary material Table S1.

Compound **2**: $[\alpha]_D^{24}$: 57.8 °C (c = 1.00, MeOH); HR-ESI-MS: *m/z* 579.2273 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR data (DMSO-d_6, 100 MHz), shown in Supplementary material Table S1.

3.5. Acid hydrolysis

Solution of compound **1** (8 mg) and **2** (8 mg) in MeOH-HCl (1:1) was heated in a water bath at 90 °C for 2 h. After cooling, the solution was diluted to 20 mL with water and then extracted with CH_2Cl_2 (20 mL × 2) (Yang et al. 2014). Then, the water layer was neutralized with 1 M aqueous KOH. After concentration, each layer was subjected to silica gel thin layer chromatography (TLC), together with the standard samples. N-BuOH-AcOH-H_2O and $CHCl_3$-MeOH-H_2O were used as the developing solvents and O-phthalic acid-aniline was used as the detection reagent. Glucose and xylose were detected. The reduced sugars were dissolved in 0.5 mL pyridine, and then 0.5 mL hexamethyl disilazane and 0.2 mL trimethylchlorosilane were added and the mixture was heated at 20 °C for 15 min. After that the sample was extracted with H_2O (2 mL). Each aqueous layer was examined by GC, H_2 flame ionization detector. The column temperature was programmed from 60 to 260 °C at a rate of 10 °C/min. Nitrogen was used as carrier gas at 1.5 mL/min. The injector and detector temperature were at 280 °C and the injection volume was 1 μL with the split ratio being 10:

1. The standard monosaccharides were subjected to the same reaction and GC analysis under the same condition. The derivatives of D-glucose and D-xylose were detected by comparison of its retention times with those of the standard sample.

3.6. Antibacterial activity

The antibacterial activities of compound **1** and **2** were evaluated by determining the minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in vitro. The MIC of compound **1** and **2** for the isolated bacterial strains were determined by 96 well-plates dilution method as previously described with as light modification (Shu et al. 2016). Briefly, bacterial strains were grown on Mueller-Hinton (MH)

agar plates and suspended in MH broth. The inoculum suspensions were prepared from 6 h broth cultures incubation and adjusted to obtain a 0.5 McFarland standard turbidity and were then diluted 1000-fold with the respective medium to the concentration of 1.5×10^5 CFU/Ml (Lu et al. 2011). Two folds serial diluted concentrations of compound **1** and **2** were added in MH broth ranging from 0.01 to 5 mg/mL. To adjust the interference by plant pigments, a parallel series of mixtures with uninoculated broth was prepared. The bacterial suspensions were aerobically incubated for 18 h at 37 °C. Triplicate samples were performed for each test concentration. MIC was defined as the lowest concentration inhibiting visible growth. The MBC determination was carried out by spreading 0.1 mL of the cultures in each well without visible growth onto sample free MH agar and incubated for 18 h. MBC was considered as the highest dilution at which almost bacterial inoculum was killed. The experiments were performed in triplicate.

Disclosure statement

The authors declare that there are no conflicts of interest.

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