New sesquiterpenoids with COX-2 inhibitory activity from the medical plant Physalis alkekengi L. var. franchetii

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ABSTRACT

Phytochemical investigations on Physalis alkekengi L. var. franchetii, a widespread traditional Chinese medicine, led to the isolation and identification of three new sesquiterpenoids physalisitins A–C (1–3). Their structures were elucidated by NMR and HRESIMS analysis, and their absolute configurations were determined by quantum chemical NMR and ECD calculations, as well as by comparing their optical rotation values with those known analogues. All of the isolated compounds were evaluated for their cyclooxygenase-2 (COX-2) inhibitory activity. Compounds 1–3 dose-dependently inhibited the COX-2 enzyme with IC50 values of 3.22 ± 0.25, 6.35 ± 0.84, and 11.13 ± 1.47 μM, respectively.

1. Introduction

Physalis alkekengi L. var. franchetii (Mast.) Makino, a member of the genus Physalis, is a famous medicinal plant which was widely distributed in China [1]. This plant could be used as traditional Chinese medicine for treatment of prostatitis, tussis, pharyngalgia, dysentery, difficulty in micturition and jaundice [2]. Numerous bioactive components, such as steroids (physalins, neophysalin and withanolides) [3–8], sesquiterpenes and norsesquiterpenes [9,10], flavonoids [11,12] and alkaloids [13] were isolated from this genus, and some of which showed considerable anti-cancer [14,15], anti-inflammatory [16], inhibit diabetes [17], antioxidant [18], and antimicrobial activities [19]. Anti-inflammatory active constituents from this genus have been reported and caught our attention. For example, the new norsesquiterpenes physanosides A and B displayed strong inhibition on lipopolysaccharide-induced NO release by macrophages with IC50 values of 9.93 and 7.31 μM, respectively [10], the seco-steroid physalin E exhibited a potent anti-inflammatory property on experimental dermatitis in mice [16].

To search for new bioactive components from this herb, a systematic phytochemical study was performed. As a result, three previously undescribed sesquiterpenoids, physalisitins A–C (1–3), were obtained and identified from the EtOAc extract of the dried persistent calyx of P. alkekengi var. franchetii. Their structures were identified by detailed interpretation of the one-dimension (1D) and two-dimension (2D) nuclear magnetic resonance (NMR), high-resolution electrospray ionization mass spectroscopy (HRESIMS) data, and their absolute configurations were determined by quantum chemical NMR and ECD calculations, as well as by comparing their optical rotation values with those known analogues. Compounds 1–3 represented rarely occurring sesquiterpene structures compared to those reported ones and their derivatives (4–7) obtained from this genus [9,10]. In terms of bioactivity, compounds 1–3 showed different levels of inhibitory activities on COX-2. Herein, we described the isolation, structural elucidation and bio-activity measurements of compounds 1–3.

2. Experimental

2.1. General experimental procedures

Optical rotation was acquired in CHCl3 on Jasco P-1020 polarimeter at room temperature. UV (Ultraviolet) spectra were recorded on V-550 UV/vis spectrophotometer. IR (Infrared) spectra were determined by a
Jasco FT/IR-480 plus Fourier Transform infrared spectrometer using KB pellet. HR-ESI-MS data were measured on an Agilent 6210 ESI/TOF mass spectrometer. Nuclear magnetic resonance (1D and 2D NMR) spectra were measured on Bruker AV-300 spectrometers. Thin-layer chromatography (TLC) analyses were carried out using pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Qingdao, China). Column chromatographies were performed on silica gel (200–400 mesh, Qingdao Haiyang Chemical Co.), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) and reverse-phase C18 silica gel (Merck, Darmstadt, Germany). All solvents used in column chromatography and high-performance liquid chromatography (HPLC) were of analytical (Sinopharm Chemical Reagent Co., Shanghai, China) grade and chromatographic grade (Fisher Scientific, NJ, USA), respectively.

2.2. Plant material

The dried persistent calyx of P. alkekengi was collected in Sichuan province, People’s Republic of China, in August 2013. The persistent calyx was authenticated by Prof. Ren-Wang Jiang (Jinan University). A voucher specimen (No. 20130831) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University.

2.3. Extraction and isolation

The dried persistent calyx (30 kg) was extracted three times with 70% ethanol at 80 °C. The ethyl alcohol solution was combined, and then evaporated under reduced pressure to afford a crude residue (1300 g), which was suspended in water and partitioned with petroleum ether, EtOAc and n-BuOH to eventually result in petroleum ether, EtOAc fraction, n-BuOH fraction and water soluble, respectively.

The EtOAc fraction (290 g) was subjected to silica gel (200–300 mesh), eluted with chloroform-methanol (30:1, 15:1, 10:1, 5:1, 3:1 and 1:1) to give 11 fractions (Fr.1 to Fr.11). Fr. 6 (7.1 g) was further purified by preparative HPLC using CH3CN-H2O (60:40) as the eluent to yield 1 (1.1 mg). Fr. 6e was purified via preparative HPLC with a MeOH-H2O (55:45) as the eluent, and further purified by CC on Sephadex LH-20 eluting with methanol to give 2 (5.7 mg). Fr. 7 (6.8 g) was further separated by CC over RP-18 eluting with a MeOH-H2O gradient (1:9 to 1:0) to yield 6 fractions (Fr. 6a−6f). Fr. 7b was further purified by Sephadex LH-20 eluting with chloroform-methanol (1:1), and then purified by preparative HPLC CH3CN-H2O (55:45) to yield 3 (14.6 mg).

2.4. Spectral data of new compounds

Physalisin A (1): yellow oil; [α]D25 = −11.3° (c 0.8, CHCl3); UV (MeOH)λmax: 242 (0.47) nm; ECD (0.50 mg/mL, CHCl3) λmax (Δε) 229 (−4.78), 247 (+1.79) nm; HRESIMS m/z = 275.1605 ([M + Na]+); calcd for C15H24O3Na: 275.1623, ESIMS: m/z 252 [M + H]+, 274 [M + Na]+; 3H and 13C NMR data, Table 1. (See Fig. 1.)

Physalisin B (2): colorless oil; [α]D25 = −26° (c 0.8, CHCl3); UV (MeOH)λmax: 242 (0.58), 273 (0.36) nm; HRESIMS m/z = 259.1695 ([M + Na]+); calcd for C15H22O3Na: 259.1674, ESIMS: m/z 237 [M + H]+, 259 [M + Na]+; 1H and 13C NMR data, Table 1.

Physalisin C (3): yellow oil; [α]D25 = −56° (c 0.8, CHCl3); UV (MeOH)λmax: 240 (0.43), 267 (0.38) nm; HRESIMS m/z = 257.1515 ([M + Na]+); calcd for C15H22O3Na: 235.1653, ESIMS: m/z 235 [M + H]+, 257 [M + Na]+; 1H and 13C NMR data, Table 1.

2.5. Bioassays

Compounds 1–3 were evaluated for COX-2 inhibitory activity using an enzyme immunoassay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI). The EIA assay was performed with aspirin as a positive control. According to the directions, the reaction mixtures were prepared COX-2 and Tris-HCl buffer, and addition of arachidonic acid initiated the reaction. The reaction was terminated by adding HCl after 2 min, and PGE2 was quantitated by ELISA method. The tested compounds were dissolved in DMSO, following transfer to a 96-well plate coated with a mouse anti-rabbit IgG, the tracer prostaglandin acetylcholine esterase and primary antibody (mouse anti PGE2) were added. Then plates were incubated at room temperature overnight, reaction mixtures were removed, and wells were washed. Ellman’s reagent was added to each well and the plate was incubated for about 1 h, until the control wells yielded an OD = 0.3–0.8 at 412 nm. Results were expressed as a percentage relative to a control (solvent-treated samples). All determinations were performed in duplicate, and values generally agreed within 10%.

The cytotoxicity of compounds 1–3 was evaluated against DU145, PC3 and LCNP cancer cell lines using the MTT method [20,21]. Briefly, cells were plated onto 96-well plates at 3 × 104 cells per well for DU145 and PC3 cell lines and 5 × 103 cells per well for LCNP cell lines. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin and streptomycin in 37 °C in a 5% CO2 humidified atmosphere. Following incubation for 48 h, 20 μL of the MTT solution [5 mg/mL in phosphate buffered saline (PBS)] was added to each well, and the cells were further incubated for 4 h. Then the medium was removed and replaced by 150 μL of DMSO in each well to dissolve the formazan crystals. The relative cell viability was determined by measuring the optical densities at 570 nm on microplate reader, and was expressed as a percentage relative to the control. The experiments were performed three times, each in triplicate. Taxol was used as a positive control [22].

2.6. Computational section

Conformational searches were performed via the molecular mechanics using MM+ method in HyperChem 8.0 software, and the geometries were further optimized at PBE0/6–311 + G (2d,2p) level via Gaussian 09 software [23] to give the energy-minimized conformers. Then, the optimized conformers were subjected to the calculations of NMR and ECD spectra using TDDFT at B3LYP/TZVP; solvent effects of the MeCN solution were evaluated at the same DFT level using the SCRF/PCM method.

3. Results and discussion

Compound 1 was obtained as yellow oil. HRESIMS ion at m/z 275.1605 [M + Na]+ indicated its molecular formula as C15H24O3 (calculated for C15H22O3Na, 275.1623) with four degrees of unsaturation. The 13C NMR and DEPT spectroscopic data (Table 1) along with HSQC spectra revealed the presence of 15 carbon atoms, which were clarified into four quaternary carbons (δ 40.1, C-6; δ 72.6, C-10; δ 76.4, C-1 and δ 148.5, C-2), including one olefinic carbon at δ 148.5 and two oxygenated carbon at δ 76.4 (C-1) and 72.6 (C-10); two olefinic methines at δ 131.4 (C-4) and 131.4 (C-3) as well as two oxygenated methines at δ 77.1 (C-5) and 78.3 (C-9); three methylenes (δ 21.8, C-8; δ 28.5, C-7 and δ 113.5, C-13) including one olefinic carbon at δ 113.5; and four methyl signals at δ 25.2 (C-11), 25.4 (C-12), 19.4 (C-14), and 17.1 (C-15). A tri-substituted pyran moiety was established from the 1H−1H COSY signals (Fig. 2) of the H-7/H-8, H-8/H-9 correlations as well the HMBC correlations of H-7/H-8, H-8/H-9 and H-9 to C-7. The HMBC correlations of H-3, H-4, and H-5 were observed in the HMBC spectrum of compound 1. The 1H−1H COSY correlations of H-2/C-4, H-2/C-5, H-2/C-6, H-2/C-7, H-2/C-8, H-2/C-9, H-3/C-4, H-3/C-5, H-3/C-6, H-3/C-7, H-3/C-8, H-3/C-9, H-4/C-5, H-4/C-6, H-4/C-7, H-4/C-8, H-4/C-9, H-5/C-6, H-5/C-7, H-5/C-8, H-5/C-9, H-6/C-7, H-6/C-8, H-6/C-9, H-7/C-8, H-7/C-9, H-8/C-9, H-9/C-10, H-10/C-11, H-10/C-12, H-11/C-12, H-12/C-9 revealed a gem-dimethyl moiety (C-10, C-11 and C-12) connected to C-9. The isopropanol-substituted pyran ring was further confirmed by NMR data comparison with those of paspaline [24]. The 1H−1H COSY correlations between H-3, H-4, and H-5 and the key HMBC correlations of H-2/C-4, C-6, H-1/C-3, C-5 and H-5/C-1, C-3 revealed the existence of a cyclohexene moiety. The pyran and cyclohexene moieties were linked.
together by the key HMBC correlations of H-5/C-7, C-9, H-7/C-1, C-5, H-8/C-6 and H-9/C-5. Combined with the HMBC correlations of H-3/C-13, H-13/C-1, C-3, H-14/C-2, C-6 and H-15/C-1, C-5, C-7, the planar structure of compound 1 was finally generated. The relative configuration of 1 was assigned by analysis of NOESY data (Fig. 3). The key NOE correlation between Me-12 and Me-14 indicated them on the same side of the molecule, while the mutual NOE correlations between H-5, H-9, and Me-15 suggested them on the opposite face.

To confirm the planar structure and relative configuration of compound 1, we tried to crystallize it for an X-ray single crystallographic analysis but failed at various conditions. Alternatively, the 1H and 13C NMR data of 1 were tentatively predicted by quantum chemical calculations using the gauge-independent atomic orbital (GIAO) method at PBE0/6–311++G (2d, 2p) level via Gaussian 09 software [23]. The calculated 1H NMR data (Table S1) with a largest deviation and corrected mean absolute deviation (CMAD) of 0.18/0.07 ppm, and the calculated 13C NMR data (Table S1) with a largest deviation and CMAD of 3.4/1.34 ppm (in CD3OD) for 1 were in good agreement with the experimental data (Table 1), which further corroborated the structure of 1.

The absolute configuration of compound 1 was studied by the TDDFT-ECD calculation. The ECD spectrum of compound 1 exhibited negative CE at 229 nm and positive CE at 247 nm, which matched well with that of the calculated (1S, 5S, 6S, 9S)-1 (Fig. 4).

Compound 2 was isolated as colorless oil. The molecular formula of 2 was assigned as C15H24O2 based on its HRESIMS ion peak at m/z 259.1695 (calcd for C15H24O2Na, 259.1674). Interpretation of the 1H and 13C NMR data (Table 1) indicated the presence of 15 carbons, which were clarified into five quaternary carbons, three methines, two methylenes, and five methyls. Based on the 1H-1H COSY correlation (Fig. 2) between H-1(δ 6.87) and H-2 (δ 6.83) and the HMBC correlations (Fig. 2) between H-1/C-3 and C-5, H-2/C-4, C-6 and C-13, H-13/C-2 and C-4, H-14/C-3 and C-5, and the correlations between H-15/C-4 and C-6 revealed a 1,2,3-trimethyl substituted (C-13, C-14 and C-15) aromatic moiety. The 1H-1H COSY correlations of H-7, H-12, between H-11 and C-9, C-12, between H-12 and C-9, C-11 revealed the existent of a hydrocarbon chain moiety with a gem-dimethyl in the terminal, thus was further confirmed by NMR data comparison with those of (R)-2-methyl-5-phenylpentane-2,3-diol [25]. The two moieties were linked by the key HMBC correlations of H-1/C-7 as well as between H-7/C-1 and C-5. Thus, the planar structure of compound 2 was elucidated as shown. The absolute configuration of 2 was studied by the

Table 1

| No. | 1        | 2        | 3        |
|-----|----------|----------|----------|
|     | δ_H (Mult, J in Hz)a | δ_C, Typeb | δ_H (Mult, J in Hz)a | δ_C, Typeb | δ_H (Mult, J in Hz)a | δ_C, Typeb |
| 1   | 76.4, C  | 6.87 (1H, d, 7.8) | 127.5, CH  | 6.84 (1H, s) | 134.2, CH  |
| 2   | 148.5, C | 6.83 (1H, d, 7.8) | 134.0, CH  | 6.84 (1H, s) | 134.0, CH  |
| 3   | 131.4, CH| 5.69 (1H, br d, 10.2) | 135.9, C  | 2.71 (1H, m) | 34.7, CH2  |
| 4   | 77.1, CH | 4.27 (1H, br s) | 139.1, C  | 2.71 (1H, m) | 34.7, CH2  |
| 5   | 40.1, C  | 128.0, CH  | 135.0, C  | 2.71 (1H, m) | 34.7, CH2  |
| 6   | 28.5, CH2| 1.28 (1H, m) | 3.22 (1H, ddd, 13.7, 10.2, 6.6) | 32.4, CH2  | 75.5, C    |
| 7   | 21.8, CH2| 1.37 (1H, m) | 1.50 (1H, m) | 33.5, CH2  | 2.68 (2H, m) | 24.4, CH2  |
| 8   | 1.39 (1H, m) | 1.42 (1H, m) | 1.79 (1H, m) | 79.1, CH  | 1.73 (1H, m) | 28.1, CH2  |
| 9   | 78.3, CH  | 3.27 (1H, dd, 11.1, 2.4) | 2.03 (1H, m) | 72.6, C  | 73.7, C    | 135.4, C   |
| 10  | 25.2, CH2| 1.13 (3H, s) | 1.12 (3H, s) | 24.9, CH4  | 76.1, C    |
| 11  | 25.4, CH2| 1.16 (3H, s) | 1.15 (3H, s) | 25.7, CH2  | 1.30 (3H, s) | 24.8, CH2  |
| 12  | 113.5, CH2 | 5.01 (1H, br s), 5.21 (1H, br s) | 2.20 (3H, s) | 20.8, CH2  | 1.30 (3H, s) | 24.8, CH2  |
| 13  | 19.4, CH2 | 1.29 (3H, s) | 2.21 (3H, s) | 16.0, CH2  | 2.17 (3H, s) | 19.6, CH2  |
| 14  | 17.1, CH3 | 1.22 (3H, s) | 2.15 (3H, s) | 15.7, CH2  | 2.18 (3H, s) | 19.7, CH2  |
| 15  | 25.2, CH2 | 25.4, CH2  | 1.13 (3H, s) | 24.9, CH2  | 76.1, C    |
|     | 25.7, CH2| 1.16 (3H, s) | 1.15 (3H, s) | 25.7, CH2  | 1.30 (3H, s) | 24.8, CH2  |
| 16  | 113.5, CH2 | 5.01 (1H, br s), 5.21 (1H, br s) | 2.20 (3H, s) | 20.8, CH2  | 1.30 (3H, s) | 24.8, CH2  |
| 17  | 156.7, C  | 128.0, CH  | 135.0, C  | 2.71 (1H, m) | 34.7, CH2  |
| 18  | 128.0, CH  | 135.0, C  | 2.71 (1H, m) | 34.7, CH2  |

Fig. 1. Structures of the new compounds 1–3 and other sesquiterpene/norsesquiterpenes obtained from P. alkekengi previously reported [9,10].
The observed rotation of 2 (\([\alpha]_D^{25} = -26.0^\circ (c 0.80, CHCl_3)\)) was in reasonable agreement with literature value of \((S)-3\)-methyl-1-phenylbutane-2,3-diol \([\alpha]_D^{25} = -55.1^\circ (c 0.86, CHCl_3)\), leading to the absolute configuration of compound 2 being determined as \(S\).

value of optical data comparison with those analogues of \((S)-3\)-methyl-1-phenylbutane-2,3-diol and \((R)-2\)-methyl-5-phenylpentane-2,3-diol \([25]\). The observed rotation of 2 (\([\alpha]_D^{25} = -26.0^\circ (c 0.80, CHCl_3)\)) was in reasonable agreement with literature value of \((S)-3\)-methyl-1-phenylbutane-2,3-diol (\([\alpha]_D^{25} = -55.1^\circ (c 0.86, CHCl_3)\)), leading to the absolute configuration of compound 2 being determined as \(S\).

The molecular formula of 3 was determined to be \(C_{15}H_{22}O_2\) on the basis of HRESIMS ion peak at \(m/z\) 257.1515 [M + Na]+ (calcd for \(C_{15}H_{22}O_2Na\), 257.1518), indicating four degrees of unsaturation. The \(^1\)H NMR spectrum (Table 1) displayed signals for two aromatic protons in the downfield region, indicating the presence of a substituted phenyl group. On the other hand, the HMBC correlations (Fig. 2) from H-1 to C-9, C-14, from H-4 to C-6, C-15, and C-4, and from H-9 to C-1, C-5, from H-6 to C-4, C-10, and from H-14 to C-1, C-3, from H-15 to C-2, C-4 suggested the connectivity between the phenyl group with C-6, C-9, C-14, C-15. The \(^1\)H-\(^1\)H COSY correlation (Fig. 2) between H-8 and H-9 and the key HMBC correlations from H-6 to C-8, C-10, from H-8 to C-6, C-10, from H-9 to C-5, C-7 suggested the presence of a six-membered ring moiety. Combined the HMBC correlations from H-6 to C-11, from H-8 to C-11, and from H-12 and H-13 to C-7, and the downfield shift of C-7 and C-11, the planar structure of 3 was unambiguously determined. The absolute configuration of 3 was also determine by the value of optical data comparison with the analogue of \((R)-(−)-2(1\text{-hydroxy-methyl})ethyl-1,2,3,4-tetrahyfro-2-naphthol \([26]\). The observed rotation of 3 (\([\alpha]_D^{20} = -56.0^\circ (c 0.80, CHCl_3)\)) was in agreement with that of \((R)-(−)-2(1\text{-hydroxy-methyl})ethyl-1,2,3,4-tetrahyfro-2-naphthol (\([\alpha]_D^{20} = -33.3^\circ (c 1.25, CHCl_3)\)), indicated the absolute configuration of 3 should be \(R\).

From a biogenetic point of view, compounds 1–3 might be derived from eudesmane-type sesquiterpenoids. Briefly, compound 1 could be biosynthesized by cyclization of the intermediate (II), which is derived from \(\gamma\)-eudesmol \([27]\) by the ring-B fractured and further oxidation (Scheme 1). Compounds 2 and 3 could be derived from the methyl rearranged production of occidentalol \([27,28]\), while compound 2 also went through an open-loop reaction in the process (Scheme 1). Alternatively, a different origin of compounds 1–3 for the methyl substitution arise from a humulane intermediate is also possible \([29]\), after a series of complex rearrangements and transannular cyclization of 6,9-Diiso-zerumbone afforded a cyclopropane intermediate, and farther oxidation or ring opening forms the structure of 1–3.

Considering the pronounced treatment effect of some the genus Physalis plants on inflammation, compounds 1–3 were tested for COX-2 inhibitory activity using an enzyme immunoassay (EIA) kit, with aspirin as a positive control. All of them showed moderate inhibitory activities (Table 2). In addition, the inhibitory activities of compounds 1–3 were evaluated on prostate cancer cells (DU145, PC3 and LNCaP). Results showed that all of them only exhibit weak activities against these cells with IC\(_{50}\) values more than 50 μM.

4. Conclusion

Three new sesquiterpenoids were isolated and identified from the persistent calyx of Physalis alkekengi L. var. franchetii. Their structures
were elucidated by NMR, HRESIMS analysis, quantum chemical NMR and ECD calculations, as well as by comparing their optical rotation values with those known analogues. All of the isolated sesquiterpenoids were tested for COX-2 inhibitory activity and cytotoxic effects. Results showed that compounds 1–3 dose-dependently inhibited the COX-2 enzyme with IC_{50} values of 3.22 ± 0.25, 6.35 ± 0.84, and 11.13 ± 1.47 μM, respectively. Cytotoxic assay showed that these sesquiterpenoids only exhibit weak activities on tumor cells (DU145, PC3 and LNCaP) with IC_{50} values more than 50 μM.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix B. Supplementary data

The followings are available online at www.///, HRESIMS, 1D and 2D NMR spectra, UVs and ECDs of compounds 1–3. Supplementary data to this article can be found online at [https://doi.org/10.1016/j.fitote.2020.104470].

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Scheme 1. Proposed biosynthetic pathway for compounds 1–3.
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