Chemical Constituents of *Pteris ensiformis* (Postprint)

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Abstract

Pteris insignis is widely distributed in Guizhou with abundant resources, and has the effects of clearing heat and promoting diuresis, as well as activating blood and reducing swelling. To date, no literature reports on its chemical constituents and biological activities have been published. This paper investigates the chemical constituents of Pteris insignis, aiming to elucidate the material basis of this plant, search for related active constituents and lead compounds, and lay a scientific foundation for the rational utilization of its resources. This study used α-tocospirone (1), cyclolaudenol (2), (2S,3S)-pterosin C (3), (2R,3S)-pterosin C (4), pterosin B (5), pterosin F (6), α-ionone'), ficusol (9), palmitic acid (β-sitosterol (18). All compounds were isolated from this plant for the first time.

Full Text

Preamble

Chemical Constituents from *Pteris insignis*

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Abstract

Pteris insignis is widely distributed and abundant in Guizhou Province, where it has been traditionally used for clearing heat, promoting diuresis, activating blood circulation, and reducing swelling. To date, no studies on its chemical constituents or biological activities have been reported in the literature. This study investigates the chemical composition of *P. insignis* to elucidate its material basis, identify bioactive constituents and lead compounds, and provide a

scientific foundation for the rational utilization of this resource. The aerial parts of *P. insignis* were extracted with 95% methanol, and the chemical constituents were isolated and purified using chromatographic techniques including silica gel, MCI gel CHP 20P, YMC gel ODS-A-HG, and Sephadex LH-20. Structures were elucidated based on spectroscopic data analysis. Eighteen compounds were isolated and identified as (-)-α-tocospirone (1), cyclolaudenol (2), (2S,3S)-pterosin C (3), (2R,3S)-pterosin C (4), pterosin B (5), pterosin F (6), α-ionone (7), sauropunol C/D (8/8'), ficusol (9), palmitic acid (10), 2-dodec-2-enyl-succinic acid dimethyl ester (11), methyl-9-phenyl-10-hydroxyoctadecanoate (12), hexadecanoic acid-2,3-dihydroxy-propyl ester (13), methyl elaidate (14), (Z,Z)-9,12-octadecadienoic acid methyl ester (15), linolenic acid methyl ester (16), daucosterol (17), and β-sitosterol (18). All compounds were isolated from this plant for the first time.

Keywords: *Pteris*; *Pteris insignis*; chemical constituents; isolation and purification; structure identification

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Pteris insignis belongs to the family Pteridaceae and genus *Pteris* L., and is documented in several botanical monographs including *Icones Filicum Sinicarum*, *Pteridophyte Flora of Guizhou*, and *Flora of Lycophytes and Ferns of Guizhou* (Qin, 1930; Wang & Wang, 2001; Wang & Pan, 2018). The plant has been traditionally used for clearing heat, promoting diuresis, activating blood circulation, and reducing swelling. Internally, it is used to treat dysentery, jaundice, stranguria, sore throat, and scrofula, while external application treats rheumatic bone pain and traumatic injuries (Pan, 2012). In Guizhou's ethnic minority regions, the whole herb is commonly used to treat sore throat and various swellings in the neck, nape, and jaw areas. In recent years, based on ethnomedicinal investigations, our research group has studied the chemical constituents of related species including *Pteris henryi* (Li et al., 2015), *Pteris deltodon* (Li et al., 2016), and *Pteris cretica* (Zou et al., 2019), discovering a series of active constituents with anti-HIV, antitumor, and antimicrobial activities. To continue searching for bioactive constituents and lead compounds from this genus, we

investigated the chemical composition of *P. insignis*, isolating 18 compounds from its aerial parts (Figure 1 [Figure 1: see original paper]): (-)-α-tocospirone (1), cyclolaudenol (2), (2S,3S)-pterosin C (3), (2R,3S)-pterosin C (4), pterosin B (5), pterosin F (6), α-ionone (7), sauropunol C/D (8/8'), ficusol (9), palmitic acid (10), 2-dodec-2-enyl-succinic acid dimethyl ester (11), methyl-9-phenyl-10-hydroxyoctadecanoate (12), hexadecanoic acid-2,3-dihydroxy-propyl ester (13), methyl elaidate (14), (Z,Z)-9,12-octadecadienoic acid methyl ester (15), linolenic acid methyl ester (16), daucosterol (17), and β-sitosterol (18). All compounds were isolated from this species for the first time.

Materials and Methods

Instruments, Reagents, and Plant Material

Instruments: JEOL 5973 MSD mass spectrometer (Agilent, USA); 600 MHz Bruker Avance NEO NMR spectrometer (Bruker, Germany); 500 MHz WNMR-I NMR spectrometer (Wuhan Zhongke Oxford Spectrum Technology Co., Ltd.); Buchi R210 rotary evaporator (Buchi, Switzerland); J209A-4 plant pulverizer (Henan Huanghua Qijiawu Scientific Instrument Factory); Mettler-Toledo electronic balance (Mettler-Toledo, Switzerland); Sephadex LH-20 dextran gel (Pharmacia, Sweden); ZF-7 darkroom three-purpose UV analyzer (Shanghai Jiapeng Technology Co., Ltd.); MCI GEL CHP 20P medium-pressure chromatography gel (75–150 m, Mitsubishi Chemical, Japan); YMC gel ODS-A-HG C18 reversed-phase material (100–20/45 m, YMC, Japan); preparative TLC plates and column chromatography silica gel (GF254 and 200–300 mesh, Qingdao Marine Chemical Co., Ltd.).

Reagents: Deuterated solvents were used for NMR analysis; all other reagents were industrial grade and redistilled before use.

Plant Material: *Pteris insignis* was collected in Libo County, Guizhou Province in October 2016 and identified by Professor Junhua Zhao of Guizhou University of Traditional Chinese Medicine. A voucher specimen (No. 20161003) is deposited in the Key Laboratory of Miao Medicine at Guizhou University of Traditional Chinese Medicine.

Extraction and Partitioning

Dried powdered fronds of *P. insignis* (24.7 kg) were extracted with 95% methanol by maceration at room temperature to yield 2.8 kg of crude extract. The extract was suspended in water and successively partitioned with petroleum ether and ethyl acetate to obtain an ethyl acetate fraction (335.61 g). This fraction was subjected to MCI column chromatography eluted with a methanol-water gradient (1:9$→$9:1) to afford four polarity fractions: A (25.42 g), B (27 g), C (80 g), and D (78.6 g).

Isolation and Purification

Fraction A was separated by C18 column chromatography using a methanol-water gradient (1:9$→$9:1) to give four subfractions (A1–A4). Subfraction A1 was subjected to silica gel column chromatography eluted with dichloromethane-methanol (19:1, 9:1, 4:1) to yield compounds 8/8' (136 mg) and compounds 3 and 4 (22 mg). Subfraction A2 was purified by silica gel column chromatography with petroleum ether-ethyl acetate (1:1) to afford compound 9 (7 mg). Subfraction A3 was separated on silica gel eluted with petroleum ether-ethyl acetate (19:1, 9:1, 5:1) to give compound 17 (13 mg). The mother liquor of subfraction A4 was chromatographed on silica gel with petroleum ether-ethyl acetate (49:1, 19:1, 9:1) to yield compound 11 (23 mg).

Fraction B was subjected to C18 column chromatography with a methanol-water gradient (1:9$→$9:1) to afford three subfractions (B1–B3). Subfraction B1 was purified by silica gel column chromatography eluted with petroleum ether-ethyl acetate (7:3) to give compound 7 (13 mg). Subfraction B2 was separated on silica gel with petroleum ether-ethyl acetate (50:1, 9:1, 1:1) to yield compounds 5 (55 mg), 6 (300 mg), and 13 (9 mg). Subfraction B3 was chromatographed on silica gel eluted with petroleum ether-ethyl acetate (100:1, 50:1, 9:1) to afford compound 16 (44 mg).

Fraction C was allowed to stand, during which needle-like crystals precipitated. Repeated recrystallization from methanol gave compound 18 (3.2 g). The mother liquor of fraction C was subjected to silica gel column chromatography eluted with petroleum ether-ethyl acetate (7:3, 3:2) to yield compound 14 (583 mg).

Fraction D was separated by silica gel column chromatography with petroleum ether-ethyl acetate (20:1, 9:1, 7:3, 1:1) to afford two subfractions (D1 and D2). Crystals precipitated from the 9:1 eluate and were recrystallized to give compound 10 (1.1 g). Subfraction D1 was purified by silica gel column chromatography eluted with petroleum ether-ethyl acetate (4:1) to afford compound 15 (205 mg) and a further subfraction D1', which was purified by gel column chromatography to yield compounds 1 (32 mg) and 3 (12 mg). Subfraction D2 was similarly chromatographed on silica gel with petroleum ether-ethyl acetate (4:1) to give compound 12 (20 mg).

Results and Analysis

Compound 1 was obtained as a colorless oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.83 (3H, d, J = 2.3 Hz, H-18), 0.84 (3H, d, J = 2.1 Hz, H-13a), 0.86 (3H, d, J = 3.3 Hz, H-21a), 0.87 (3H, br s, H-22), 1.34 (3H, s, H-9a), 1.37 (3H, s, H-3a), 2.06 (3H, s, H-5a), 2.07 (3H, s, H-6a), 3.82 (1H, s, -OH); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 198.8 (C-1), 93.3 (C-2), 81.2 (C-3), 24.2 (C-3a), 201.7 (C-4), 142.0 (C-5), 13.1 (C-5a), 146.9 (C-6), 13.4 (C-6a), 32.0 (C-7), 36.4 (C-8), 87.1 (C-9), 25.7 (C-9a), 41.4 (C-10), 22.3 (C-11), 37.5 (C-12), 32.8 (C-13), 19.8 (C-13a), 37.5 (C-14), 24.8 (C-15), 37.4 (C-16), 32.7 (C-17), 19.7 (C-17a), 37.3 (C-18),

24.5 (C-19), 39.4 (C-20), 28.0 (C-21), 22.6 (C-21a), 22.7 (C-22). These data are consistent with literature values (Lin et al., 2003), identifying compound 1 as (-)-α-tocospirone.

Compound 2 was isolated as a white powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.33 (1H, d, J = 4.0 Hz, H-19), 0.55 (1H, d, J = 3.8 Hz), 0.81 (3H, s, H-29), 0.87 (3H, d, J = 3.5 Hz, H-21), 0.88 (3H, s, H-30), 0.96 (3H, s, H-18), 0.97 (3H, s, H-28), 1.00 (3H, d, J = 6.9 Hz, H-31), 1.64 (3H, s, H-27), 3.28 (1H, m, H-3), 4.67 (2H, br s, H-26); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 32.0 (C-1), 30.4 (C-2), 78.9 (C-3), 40.5 (C-4), 47.1 (C-5), 21.1 (C-6), 28.1 (C-7), 48.0 (C-8), 20.0 (C-9), 26.1 (C-10), 26.5 (C-11), 32.9 (C-12), 48.8 (C-13), 45.3 (C-14), 35.6 (C-15), 26.0 (C-16), 52.3 (C-17), 18.3 (C-18), 29.7 (C-19), 36.0 (C-20), 18.7 (C-21), 33.9 (C-22), 31.5 (C-23), 41.6 (C-24), 150.3 (C-25), 109.4 (C-26), 18.0 (C-27), 25.5 (C-28), 14.1 (C-29), 19.3 (C-30), 20.2 (C-31). These data are consistent with literature values (Cantillo Ciau et al., 2001), identifying compound 2 as cyclolaudenol.

Compound 3 was obtained as a white oily liquid. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.29 (3H, d, J = 7.0 Hz, H-11), 2.46 (1H, m, H-2), 2.48 (3H, s, H-12), 2.64 (3H, s, H-15), 3.01 (2H, m, H-13), 3.61 (2H, t, J = 7.0 Hz, H-14), 4.68 (1H, d, J = 5.9 Hz, H-3), 7.35 (1H, s, H-4); ^{13}C-NMR (125 MHz, CD_3OD) δ: 207.6 (C-1), 54.6 (C-2), 75.9 (C-3), 126.7 (C-4), 146.3 (C-5), 138.2 (C-6), 137.9 (C-7), 132.4 (C-8), 154.5 (C-9), 13.3 (C-10), 21.4 (C-12), 33.1 (C-13), 61.6 (C-14), 14.0 (C-15). These data are consistent with literature values (Tian et al., 2011; Ng & McMorris, 1984), identifying compound 3 as (2S,3S)-pterosin C.

Compound 4 was isolated as a white solid. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.17 (3H, d, J = 7.3 Hz, H-11), 2.46 (1H, m, H-2), 2.48 (3H, s, H-12), 2.64 (3H, s, H-15), 3.01 (2H, m, H-13), 3.61 (2H, t, J = 7.0 Hz, H-14), 5.14 (1H, d, J = 5.9 Hz, H-3), 7.35 (1H, s, H-4); ^{13}C-NMR (125 MHz, CD_3OD) δ: 210.3 (C-1), 49.7 (C-2), 70.2 (C-3), 126.7 (C-4), 138.2 (C-6), 138.1 (C-7), 132.2 (C-8), 155.1 (C-9), 10.7 (C-11), 21.4 (C-12), 33.1 (C-13), 61.6 (C-14), 14.1 (C-15). These data are consistent with literature values (Ng & McMorris, 1984), identifying compound 4 as (2R,3S)-pterosin C.

Compound 5 was obtained as colorless transparent block crystals (from ethyl acetate). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.27 (3H, dd, J = 10.7, 4.3 Hz, H-10), 2.44 (3H, s, H-12), 2.56–2.83 (2H, m, H-3), 2.68 (3H, s, H-15), 3.02 (2H, t, J = 7.5 Hz, H-13), 3.23 (1H, dd, J = 16.7, 7.8 Hz, H-2), 3.75 (2H, t, J = 7.4 Hz, H-14), 7.10 (1H, s, H-4); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 210.3 (C-1), 42.5 (C-2), 33.8 (C-3), 125.7 (C-4), 144.4 (C-5), 134.8 (C-6), 138.0 (C-7), 132.2 (C-8), 152.6 (C-9), 16.6 (C-10), 21.4 (C-12), 61.6 (C-13), 31.8 (C-14), 13.7 (C-15). These data are consistent with literature values (Chen et al., 2008), identifying compound 5 as pterosin B.

Compound 6 was isolated as a white powder. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.27 (3H, d, J = 7.3 Hz, H-10), 2.43 (3H, s, H-12), 2.56–2.84 (2H, m, H-3), 2.67

(3H, s, H-15), 3.17 (2H, t, J = 8.5 Hz, H-13), 3.23 (1H, dd, J = 16.7, 7.8 Hz, H-2), 3.53 (2H, t, J = 8.8 Hz, H-14), 7.11 (1H, s, H-4); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 210.0 (C-1), 42.5 (C-2), 33.8 (C-3), 125.9 (C-4), 143.8 (C-5), 134.5 (C-6), 137.7 (C-7), 132.2 (C-8), 153.0 (C-9), 16.5 (C-10), 21.1 (C-12), 32.2 (C-13), 42.0 (C-14), 13.6 (C-15). These data are consistent with literature values (Fukuoka et al., 1983), identifying compound 6 as pterosin F.

Compound 7 was obtained as a light yellow oily liquid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.89 (3H, s, H-12), 0.97 (3H, s, H-13), 1.39 (1H, m, H-2b), 1.63 (3H, s, H-11), 1.70 (1H, dd, J = 12.9, 6.5 Hz, H-2a), 4.25 (1H, m, H-3), 2.26 (3H, s, H-10), 2.28 (1H, overlap, H-6), 5.59 (1H, br s, H-4), 6.07 (1H, d, J = 15.8 Hz, H-8), 6.63 (1H, dd, J = 15.8, 9.6 Hz, H-7); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 35.0 (C-1), 40.7 (C-2), 66.5 (C-3), 126.4 (C-4), 132.8 (C-5), 54.3 (C-6), 147.7 (C-7), 135.4 (C-8), 198.2 (C-9), 27.0 (C-10), 22.4 (C-11), 29.7 (C-12), 27.0 (C-13). These data are consistent with literature values (Li & Jia, 2003), identifying compound 7 as α-ionone.

Compound 8/8' was isolated as a colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.05–2.25 (2H, m, H-2), 4.53–4.72 (1H, m, H-5), 5.72–5.54 (1H, m, H-1), 4.25–3.37 (3H, m, H-3, 4, 6); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 100.6 (C-1), 100.2 (C-1'), 41.5 (C-2), 40.2 (C-2'), 84.4 (C-4), 82.8 (C-4'), 74.9 (C-3), 71.8 (C-3'), 81.9 (C-5), 80.8 (C-5'), 70.9 (C-6), 70.9 (C-6'). These data are consistent with literature values (Zhang et al., 2016), identifying compound 8/8' as sauropunol C/D.

Compound 9 was obtained as a white oily liquid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 3.71 (3H, s, H-OCH_3), 3.82–3.76 (2H, m, H-3b, H-2), 3.89 (3H, s, H-OCH_3), 4.11 (1H, dd, J = 10.7, 8.5 Hz, H-3a), 6.76 (1H, dd, J = 8.1, 1.9 Hz, H-6'), 6.78 (1H, d, J = 1.7 Hz, H-2'), 6.88 (1H, d, J = 8.0 Hz, H-5'); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 173.8 (C-1), 53.5 (C-2), 64.7 (C-3), 127.3 (C-1'), 114.7 (C-2'), 145.3 (C-3'), 146.7 (C-4'), 110.5 (C-5'), 121.2 (C-6'), 52.2 (C-OCH_3), 56.0 (C-OCH_3). These data are consistent with literature values (Yi et al., 2011), identifying compound 9 as ficusol.

Compound 10 was isolated as white scales. Co-TLC with an authentic palmitic acid standard using three different solvent systems showed identical Rf values, and the mixed melting point was not depressed. EI-MS m/z: 256 $[M]^+$, 239 $[M\text{-}OH]^+$, 199, 185, 115, 73. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.89 (3H, t, J = 6.9 Hz, H-16), 1.30–1.25 (24H, m, H-4–H-15), 1.63–1.59 (2H, m, H-3), 2.31 (2H, t, J = 7.5 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 14.1 (C-1), 34.0 (C-2), 31.9 (C-3), 29.7 (C-4–C-8), 29.6 (C-9), 29.5 (C-10), 29.4 (C-11), 29.3 (C-12), 29.1 (C-13), 24.7 (C-14), 22.7 (C-15), 179.7 (C-16). These data are consistent with literature values (Lu et al., 2009), identifying compound 10 as palmitic acid.

Compound 11 was obtained as a white oily liquid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 6.7 Hz, H-15), 1.26–1.35 (14H, m, H-8–H-14), 2.03 (4H, m, H-4, 7), 2.30 (1H, t, J = 7.3 Hz, H-2b), 3.03 (1H, m, H-2a, 3),

3.66 (3H, s, H-OCH_3), 3.68 (3H, s, H-OCH_3), 4.13 (6H, dd, J = 5, 15 Hz), 5.54 (2H, m, H-5, 6); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 31.9 (C-1), 121.3 (C-2), 135.0 (C-3), 32.5 (C-4), 29.2 (C-5), 29.4 (C-6), 29.5 (C-7), 29.1 (C-8), 27.2 (C-9), 29.7 (C-10), 22.8 (C-11), 14.1 (C-12), 34.1 (C-13), 38.0 (C-14), 51.4 (C-15), 51.7 (C-16), 172.6 (C-17), 174.3 (C-18). These data are consistent with literature values (Olejniczak, 2010), identifying compound 11 as 2-dodec-2-enyl-succinic acid dimethyl ester.

Compound 12 was isolated as a white solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (3H, td, J = 6.9, 2.4 Hz, H_3-18), 1.24–1.47 (26H, m, H-4–H-7, H-12–H-17), 1.63–1.56 (2H, m, H-3), 2.05–2.09 (1H, m, H-9), 2.30 (2H, t, J = 7.5 Hz, H-2), 3.57–3.49 (1H, m, H-10), 3.67 (3H, s, H-OCH_3), 7.27–7.10 (3H, m, *p*- and *o*-aromatic H), 7.28 (2H, m, *m*-aromatic H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.3 (C-1), 34.1 (C-2), 24.9 (C-3), 22.6 (C-4), 25.1 (C-5), 27.7 (C-6), 29.0 (C-7), 29.1 (C-8), 56.0 (C-9), 82.4 (C-10), 35.7 (C-11), 29.3 (C-12), 29.5 (C-13), 29.6 (C-14), 29.7 (C-15), 31.9 (C-16), 32.6 (C-17), 14.1 (C-18), 51.4 (C-OCH_3), 127.7 (*p*-aromatic), 129.5 (*o*-aromatic), 130.9 (*m*-aromatic), 139.1 (quaternary phenyl). These data are consistent with literature values (Dailey et al., 2009), identifying compound 12 as methyl-9-phenyl-10-hydroxyoctadecanoate.

Compound 13 was isolated as a white powder. EI-MS *m/z*: 330 $[M]^+$. The fragment ion at *m/z* 239 corresponds to a hexadecanoic acid $[M\text{-}OH]^+$ fragment, and the difference between 330 and 239 matches the mass of a glycerol $[M\text{-}H]^+$ fragment. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 7.5 Hz, H-16), 1.31–1.25 (24H, m, H-4–H-15), 1.65 (2H, m, H-3), 2.35 (2H, t, J = 7.5 Hz, H-2), 3.60 (1H, dd, J = 11.4, 5.8 Hz), 3.70 (1H, dd, J = 11.4, 3.7 Hz, H-3'a), 3.93 (1H, m, H-2'), 4.21 (1H, dd, J = 11.7, 4.5 Hz, H-1'a), 4.15 (1H, dd, J = 11.7, 6.2 Hz, H-1'b); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.4 (C-1), 34.2 (C-2), 24.9 (C-3), 29.4 (C-4), 29.1 (C-5), 29.5 (C-6), 29.7 (C-7–C-12), 29.3 (C-13), 31.9 (C-14), 22.7 (C-15), 14.1 (C-16), 65.2 (C-1'), 70.3 (C-2'), 63.3 (C-3'). These data are consistent with literature values (Liu et al., 2006), identifying compound 13 as hexadecanoic acid-2,3-dihydroxy-propyl ester.

Compound 14 was obtained as a colorless oil. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 7.5 Hz, H_3-19), 1.30–1.25 (22H, m, H-4–H-7, H-12–H-18), 1.63–1.59 (2H, m, H-3), 2.03–1.99 (4H, m, H-8, 11), 2.32 (2H, t, J = 7.5 Hz, H-2), 3.67 (3H, s, H-OCH_3), 5.35–5.34 (2H, m, H-9, 10); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.3 (C-1), 34.1 (C-2), 25.0 (C-3), 27.2 (C-4), 27.2 (C-5), 29.1 (C-6), 29.2 (C-7), 29.3 (C-8), 130.0 (C-9), 129.8 (C-10), 29.3 (C-11), 29.5 (C-12), 29.5 (C-13), 29.7 (C-14), 29.8 (C-15), 31.9 (C-16), 22.7 (C-17), 14.1 (C-18), 51.4 (C-OCH_3). These data are consistent with literature values (Thao et al., 2009), identifying compound 14 as methyl elaidate.

Compound 15 was obtained as a light yellow oily liquid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.90 (3H, t, J = 7.0 Hz, H-18), 1.38–1.27 (14H, m, H-4–H-7, H-15–H-17), 1.67–1.62 (2H, m, H-3), 2.09–2.04 (4H, m, H-8, 14), 2.32 (2H, t, J = 7.6 Hz, H-2), 2.78 (2H, t, J = 6.5 Hz, H-11), 3.68 (3H, s, H-OCH_3), 5.42–5.31 (4H, m, H-9, 10, 12, 13). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.3 (C-1), 34.2 (C-

2), 25.0 (C-3), 29.0 (C-4), 29.4 (C-5), 29.4 (C-6), 29.5 (C-7), 27.1 (C-8), 129.9 (C-9), 128.0 (C-10), 25.7 (C-11), 128.4 (C-12), 130.4 (C-13), 27.3 (C-14), 29.4 (C-15), 31.6 (C-16), 22.7 (C-17), 14.2 (C-18), 51.5 (C-OCH_3). These data are consistent with literature values (Huh et al., 2010), identifying compound 15 as methyl linoleate.

Compound 16 was obtained as a light yellow oily liquid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 1.00 (3H, t, J = 9.0 Hz, CH_3, H-18), 1.41–1.26 (8H, m, H-4–H-7), 1.68–1.63 (2H, m, H-3), 2.11–2.07 (4H, m, H-8, 17), 2.33 (2H, J = 7.6 Hz, H-2), 2.82 (4H, t, J = 9.0 Hz, H-11, 14), 3.69 (3H, s, H-OCH_3), 5.41–5.34 (6H, m, H-9, 10, 12, 13, 15, 16). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 174.2 (C-1), 34.1 (C-2), 24.9 (C-3), 28.8 (C-4), 29.2 (C-5), 29.4 (C-6), 29.7 (C-7), 27.0 (C-8), 130.0 (C-9), 127.9 (C-10), 25.6 (C-11), 128.2 (C-12), 128.3 (C-13), 25.5 (C-14), 127.1 (C-15), 131.9 (C-16), 20.6 (C-17), 14.3 (C-18), 51.5 (C-OCH_3). These data are consistent with literature values (Zhang et al., 2012), identifying compound 16 as (Z,Z,Z)-9,12,15-octadecatrienoic acid methyl ester.

Compound 17 was isolated as a white powder. ^{1}H-NMR (500 MHz, C_5D_5N) δ: 0.75 (3H, s, H-18), 0.94–1.08 (15H, m, 5 × CH_3), 4.04–4.66 (5H, m, H-2'–6'), 5.14 (1H, d, J = 7.5 Hz, H-1'), 5.38–5.43 (1H, m, H-6); ^{13}C-NMR (125 MHz, C_5D_5N) δ: 37.3 (C-1), 30.1 (C-2), 71.5 (C-3), 42.3 (C-4), 140.9 (C-5), 121.7 (C-6), 31.9 (C-7), 32.0 (C-8), 50.2 (C-9), 36.7 (C-10), 21.1 (C-11), 39.2 (C-12), 39.8 (C-13), 56.6 (C-14), 24.3 (C-15), 28.4 (C-16), 56.1 (C-17), 12.0 (C-18), 19.2 (C-19), 36.2 (C-20), 19.0 (C-21), 34.0 (C-22), 26.2 (C-23), 45.8 (C-24), 29.5 (C-25), 20.0 (C-26), 18.9 (C-27), 23.2 (C-28), 11.8 (C-29), Glc: 102.4 (C-1'), 78.4 (C-2'), 78.3 (C-3'), 75.1 (C-4'), 77.9 (C-5'), 62.6 (C-6'). These data are consistent with literature values (Li et al., 2004), identifying compound 17 as daucosterol.

Compound 18 was isolated as white needle crystals (from chloroform). ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 0.68 (3H, s, H-18), 0.81 (3H, br d, J = 6.8 Hz, H-29), 0.83 (3H, t, J = 2.1 Hz, H-26), 0.84 (3H, d, J = 2.2 Hz, H-27), 0.92 (3H, t, J = 6.5 Hz, H-21), 1.01 (3H, s, H-19), 3.52 (1H, m, H-3), 5.35 (1H, d, J = 5.2 Hz, H-6); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 37.3 (C-1), 31.9 (C-2), 71.8 (C-3), 40.0 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.7 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 11.9 (C-18), 19.8 (C-19), 36.2 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.2 (C-25), 19.4 (C-26), 19.1 (C-27), 23.1 (C-28), 12.0 (C-29). These data are consistent with literature values (Liu et al., 2007), identifying compound 18 as β-sitosterol.

Discussion and Conclusion

Guizhou's unique topography and humid climate make it one of China's richest regions for pteridophytes, with 34 species of the genus *Pteris* distributed in the province (Wang & Pan, 2018), of which 18 are used medicinally (Pan, 2012). Our research group has therefore undertaken systematic studies on the chemical

constituents and bioactivities of *Pteris* species from Guizhou to discover novel bioactive compounds and provide a scientific basis for the rational utilization of these resources.

Literature surveys revealed no previous reports on the chemical constituents of *Pteris insignis*, making this study the first of its kind and thus novel and original. We isolated and identified 18 compounds from this plant, including two steroids, two triterpenoids, four sesquiterpenoids, one ionone, six aliphatic compounds, and two miscellaneous compounds. The diverse structural types enrich the chemical knowledge of the genus *Pteris* and natural products, providing valuable data and sources for related compounds.

Previous reports indicate that *Pteris* species primarily contain diterpenoids and their glycosides, sesquiterpenoids and their glycosides, and flavonoids. However, we did not isolate any diterpenoids, instead obtaining four sesquiterpenoids with a 1-indanone skeleton (containing 14 or 15 carbon atoms), which further confirms sesquiterpenoids as characteristic constituents of the genus. Notably, pterosin B is a sesquiterpenoid with demonstrated antitumor activity and cytotoxic effects against HL-60 cells (Chen et al., 2008). Our group will conduct further bioactivity screening of these sesquiterpenoids.

Additionally, literature reports show that other compounds from *Pteris* species exhibit antitumor, antiplatelet aggregation, anti-inflammatory, and antimicrobial activities (Yu et al., 2001; Gong et al., 2007). Future research will focus on targeted and prioritized investigations to discover novel compounds with significant bioactivities.

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