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ORIGINAL ARTICLE

Chemical Constituents from *Saussurea pachyneura*

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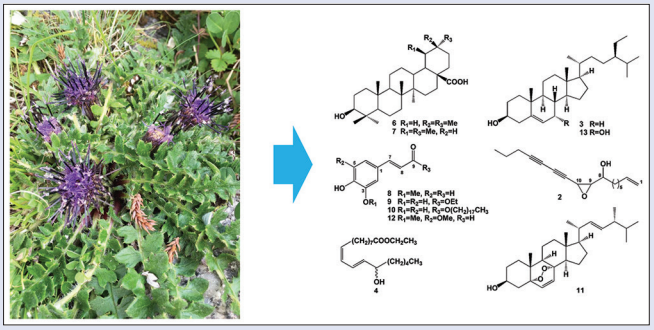
ABSTRACT

Background: Genus *Saussurea* is famous for its use in traditional Chinese medicine. **Objectives:** *Saussurea pachyneura*, a plant from genus *Saussurea*, was studied for its secondary metabolites. **Materials and Methods:** The air-dried whole plant material was extracted by ethanol. The compounds from the ethanolic extract were isolated and purified by silica gel, octadecylsilyl-silica gel, and Sephadex LH-20 column chromatography. Their structures were identified based on the spectral analysis. **Results:** A total of 13 compounds, including a new long-chain fatty acid ester (4), were isolated and identified from the ethanolic extract of *S. pachyneura*. **Conclusion:** This is the first study to report the phytochemical analysis of *S. pachyneura* and also the first study to isolate the compounds from ethanolic extract of the whole plant material of *S. pachyneura*.

Key words: Compositae, *Saussurea pachyneura*, secondary metabolites, long-chain fatty acid esters, structural identification

SUMMARY

- The phytochemical investigation of the whole plant of *Saussurea pachyneura* DC resulted in the isolation of 13 compounds
- These compounds include four phenylpropanoids (compounds 8–10, 12), three long-chain fatty acids/esters (compounds 1, 4, and 5), three steroids (compounds 3, 11, and 13), two triterpenoids (compounds 6 and 7), and one enyne (compound 2)
- Compound 4 has been identified as a new long-chain fatty acid ester
- This is the first phytochemical study on *S. pachyneura*.



Abbreviations used: NMR: Nuclear magnetic resonance; HR-ESI-MS: High-resolution electrospray ionization mass spectrometry; CC: Column chromatography

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INTRODUCTION

Genus *Saussurea* DC. (Compositae) comprises approximately 400 species worldwide, and among them, approximately 264 species are distributed in China.[1] The roots, flowers, leaves, and whole plants of many *Saussurea* species have been used as traditional Tibetan medicine in China to treat rheumatoid arthritis, traumatic injury, gynecological diseases, altitude stress, and so on.[2]

Saussurea pachyneura Franch., a perennial herb from genus *Saussurea*, is mainly distributed on the hillside, scrub, meadow, and gravel area of the Hengduan Mountains, China, at an altitude of 3285–4700 m.[1] To date, there is no phytochemical data available in the literature on *S. pachyneura*. As a continuation of our phytochemical studies on medicinal plants collected in Tibetan area,[3-7] we conducted research on *S. pachyneura*. In this study, we report the isolation and structural elucidation of the 13 compounds isolated from the whole plant of *S. pachyneura* [Figure 1], including a new compound, ethyl (9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoate (4).

MATERIALS AND METHODS

General

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV II-400 and 600 spectrometers with tetramethylsilane as an internal standard. Melting points were measured on an X-4 digital display micro melting point apparatus and uncorrected. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was acquired on a Waters Q-TOF Premier. Column chromatography (CC) was conducted using silica gel (Qingdao Marine Chemical Industry, 200–300 mesh), octadecylsilyl-silica gel (YMC, aperture 120 Å, particle size 50 μm), and Sephadex LH-20 (GE Healthcare). All reagents and solvents used in the separation and purification of compounds were of analytical grade and were purchased from local firms.

Plant material

The whole plant material of *S. pachyneura* was collected in August 2016 from Zheduo Mountain, Kangding County, Sichuan Province, China. The plant was identified by Prof. Qin-Mao Fang, Institute of Traditional Chinese medicine Medicinal Resources and Cultivation, Sichuan Academy of Chinese Medicine Science. A voucher specimen (No. SP 1608) has been deposited in the School of Life Science and Technology, University of Electronic Science and Technology of China.

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Extraction and isolation

The air-dried whole plant material of *S. pachyneura* (6 kg) was powdered and extracted with 95% aqueous ethanol at room temperature (3 × 10 L, up to 7 days). The solvent was evaporated *in vacuo* to yield ethanolic extract, which was suspended in distilled water (9 L) and then extracted with EtOAc (3 × 10 L). The EtOAc extract (90 g) was subjected to CC over silica gel (200–300 mesh, 2 kg) and eluted with a gradient solvent system (petroleum ether: EtOAc, 120:1–1:1) to yield 20 fractions (Fr. 1–20). Fraction 5 (1.1 g) was separated by silica gel chromatography (cyclohexane: acetone, 30:1) to yield 10 subfractions (Sub-Fr. 5-1–5-10). Subfraction 5-4 (86 mg) was purified by Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1) to yield compound 1 (67 mg). Subfraction 5–6 (320 mg) was separated by silica gel chromatography (cyclohexane: acetone, 50:1) and purified on Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1) to yield compound 2 (12 mg). Subfraction 5–8 (420 mg) was isolated by silica gel chromatography (cyclohexane: acetone, 50:1) and Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1) and subsequently recrystallized ($CHCl_3$:MeOH, 2:1) to yield compound 3 (51 mg). Fraction 8 (1.3 g) was isolated by silica gel chromatography (cyclohexane: acetone, 70:1), Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1), and preparative thin-layer chromatography (TLC) (cyclohexane: acetone, 5:1) to yield compound 4 (13 mg). Fraction 9 was isolated by silica gel chromatography (cyclohexane: EtOAc, 40:1–30:1) and preparative TLC ($CHCl_3$:acetone, 20:1) and then purified on Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1) to yield compound 5 (1.5 mg). Fraction 11 (1.3 g) was isolated by silica gel chromatography (cyclohexane: EtOAc, 15:1) and recrystallized (petroleum ether); the crystals were then purified by Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1) to yield compounds 6 and 7 (15 mg). Fraction 13 (1.0 g) was isolated by silica gel chromatography (cyclohexane: EtOAc, 15:1) and octadecylsilyl-silica gel chromatography (MeOH) and then purified on Sephadex LH-20 ($CHCl_3$:MeOH, 2:1) to yield compound 8 (4.2 mg). Fraction 14 (766 mg) was isolated by silica gel chromatography (cyclohexane: EtOAc, 15:1–10:1) and recrystallized (cyclohexane). Subsequently, the crystals were purified by Sephadex LH-20 ($CHCl_3$:MeOH, 2:1) to yield compound 9 (8.9 mg). Fraction 15 (1.1 g) was isolated by silica gel chromatography (cyclohexane: EtOAc, 8:1) to yield 10 subfractions (Sub-Fr. 15-1–15-10). Compound 10 (7.8 mg) was obtained from subfraction 15-5 by recrystallization (petroleum ether) and by isolation via Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1). Compound 11 (2.3 mg) was obtained from subfraction 15-8 by octadecylsilyl-silica gel chromatography (MeOH). Fraction 16 (1.0 g) was isolated by silica gel chromatography (cyclohexane: acetone, 7:1–3:1) and purified by Sephadex LH-20 CC ($CHCl_3$:MeOH, 2:1) to yield compound 12 (10 mg). Fraction 18 (2.0 g) was isolated by silica gel chromatography (cyclohexane: EtOAc, 5:1) and octadecylsilyl-silica gel chromatography (MeOH) to yield compound 13 (23.7 mg).

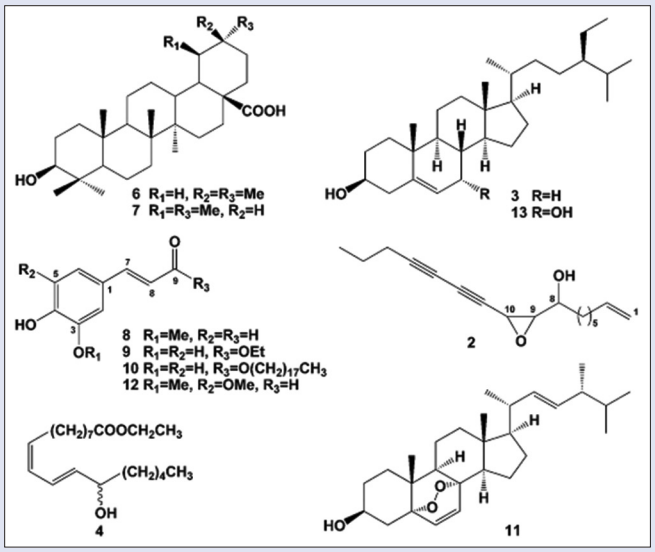

Figure 1: Structure of compounds 2–4 and 6–13 isolated from *Saussurea pachyneura*

RESULTS

New compound

Compound 4 was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{20}H_{36}O_3$ on the basis of HR-ESI-MS (*m/z*: [M + Na] + calc. 347.2562; found 347.2563) and its ^{13}C-NMR spectroscopic data, indicated three degrees of unsaturation.

Analysis of the ^{1}H and ^{13}C NMR spectra of compound 4 [Table 1] showed a set of unsaturated fatty acid ester signals, one *cis*-olefin (δ_H 5.43 [1H, dt, *J* = 7.6, 10.7 Hz], δ_C 132.8; δ_H 5.97 [1H, t, *J* = 10.9 Hz], δ_C 127.8), one *trans*-olefin (δ_H 6.48 [1H, dd, *J* = 11.0, 15.1 Hz], δ_C 125.7; δ_H 5.66 [1H, dd, *J* = 6.8, 15.2 Hz], δ_C 135.9), one oxygen-bearing methine group (δ_H 4.15 [1H, q, *J* = 6.5 Hz], δ_C 72.9), one ethoxy group (δ_H 4.12 [2H, q, *J* = 7.0 Hz], δ_C 60.1; δ_H 1.25 [3H, t, *J* = 7.0 Hz], δ_C 14.2), one ester carbonyl group (δ_C 173.9), one methyl group (δ_H 0.88 [3H, t, *J* = 6.7 Hz], δ_C 14.0), and a series of methylene groups (δ_H 2.28 [2H, t, *J* = 7.5 Hz], δ_C 34.3; δ_H 2.17 [2H, m], δ_C 27.8; δ_H 1.30–1.61 [18H, m],

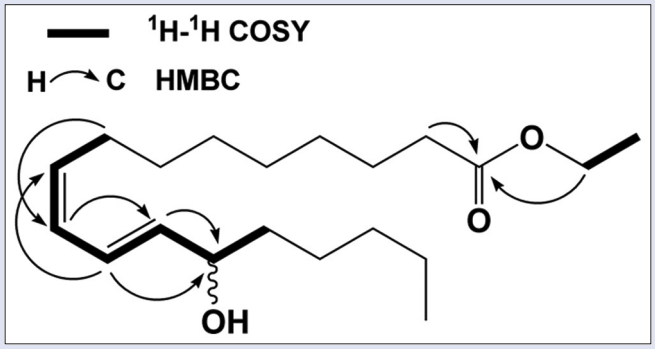

Figure 2: Selected ^{1}H-^{1}H COSY and HMBC correlation of compound 4

Table 1: ^{1}H and ^{13}C nuclear magnetic resonance data for 4 (600 and 150 MHz in $CDCl_3$, δ in ppm, *J* in Hz)

Position	δ_H (mult., *J* in Hz)	δ_c	Position	δ_H (mult., *J* in Hz)	δ_c
1	-	173.9	11	6.48 (1H, dd, 11.0, 15.1)	125.7
2	2.28 (2H, t, 7.5)	34.3	12	5.66 (1H, dd, 6.8, 15.2)	135.9
3-7, 14-17	1.30~1.61 (18H, m)	22.6~37.3	13	4.15 (1H, q, 6.5)	72.9
8	2.17 (2H, m)	27.8	18	0.88 (3H, t, 6.7)	14.0
9	5.43 (1H, dt, 7.6, 10.7)	132.8	1'	4.12 (2H, q, 7.0)	60.1
10	5.97 (1H, 5, 10.9)	127.8	2'	1.25 (3H, t, 7.0)	14.2

δ_C 37.3, 31.7, 29.4, 29.0, 29.0, 28.9, 25.1, 24.9, 22.6). A proton spin system (-CH_2-CH (OH)-CH = CH-CH = CH-CH_2-) was deduced from the ^{1}H-^{1}H COSY spectra [Figure 2]. These NMR data were similar with (9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoic acid,[8] an oxidation product of linoleic acid, except for the presence of one more ethoxy group, which indicated that compound 4 was the ethyl ester of (9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoic acid. The comparison of NMR data between compound 4 and methyl (9*Z*,11*E*,13*S*)-13-hydroxy-9,11-octadecadienoate further confirmed this deduction.[9,10] Thus, the structure of compound 4 was elucidated and named as ethyl (9*Z*,11*E*)-13-hydroxy-9,11-octadecadienoate.

Known compounds

Compound 1: Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.35 (4H, m, H-9, 10, 12, 13), 2.77 (2H, t, *J* = 6.6 Hz, H-11), 2.34 (2H, t, *J* = 7.4 Hz, H-2), 2.04 (4H, dd, *J* = 6.8, 13.6 Hz, H-8 and 14), 1.63 (2H, m, H-3), 1.25–1.31 (14H, m, 7 × CH_2), 0.88 (3H, t, *J* = 6.8 Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 180.5 (C-28), 130.2 (C-9), 130.0 (C-13), 128.0 (C-10), 127.9 (C-12), 34.1 (C-2), 31.5 (C-16), 29.6 (C-7), 29.3 (C-15), 29.1 (C-4), 29.1 (C-5), 29.0 (C-6), 27.2 (C-14), 27.2 (C-8), 25.6 (C-11), 24.6 (C-3), 22.6 (C-17), 14.0 (C-18). By comparing the NMR data of compound 1 with those reported in the literature,[11] it was identified as linoleic acid.

Compound 2: Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.81 (1H, ddt, *J* = 17.0, 10.2, 6.6 Hz, H-2), 5.00 (1H, ddd, *J* = 17.1, 3.6, 1.6 Hz, H-1), 4.93 (1H, dm, *J* = 10.1 Hz, H-1), 3.63 (1H, dt, *J* = 7.7, 5.1 Hz, H-8), 3.60 (1H, d, *J* = 4.0 Hz, H-10), 3.04 (1H, dd, *J* = 8.0, 4.0 Hz, H-9), 2.26 (2H, t, *J* = 7.0 Hz, H-15), 2.06 (2H, dd, *J* = 13.8, 6.7 Hz, H-3), 1.62 (2H, m, H-7), 1.56 (2H, q, *J* = 7.1 Hz, H-16), 1.52 (1H, m, H-6), 1.40 (4H, m, H-4 and H-5), 1.37 (1H, m, H-6), 0.99 (3H, t, *J* = 7.3 Hz, H-17). ^{13}C-NMR (100 Hz, $CDCl_3$): δ_C 139.0 (C-2), 114.2 (C-1), 81.9 (C-14)*, 71.4 (C-13)*, 71.4 (C-8), 69.4 (C-11)*, 64.3 (C-12)*, 61.6 (C-9), 45.2 (C-10), 33.7 (C-3), 33.0 (C-7), 28.9 (C-5), 28.7 (C-4), 24.6 (C-6), 21.5 (C-16), 21.2 (C-15), 13.4 (C-17). (*may be interchanged). By comparing the NMR data of compound 2 with those reported in literature,[12] it was identified and named saupachynol.

Compound 3: Colorless needle (petroleum ether). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.35 (1H, m, H-6), 3.52 (1H, m, H-3), 1.00 (3H, s, H-19), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.84 (3H, t, *J* = 7.6 Hz, H-29), 0.83 (3H, d, *J* = 7.1 Hz, H-26), 0.81 (3H, d, *J* = 6.8 Hz, H-27), 0.67 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-4), 42.3 (C-13), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-7), 31.9 (C-8), 31.6 (C-2), 29.1 (C-25), 28.2 (C-16), 24.3 (C-15), 23.0 (C-28), 21.0 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.7 (C-21), 12.0 (C-18), 11.8 (C-29). By comparing the NMR data of compound 3 with those reported in the literature,[13] it was identified as β-sitosterol.

Compound 5: White amorphous powder. HR-ESI-MS (negative) *m/z* 283.2620 [M-H]$^-$, cald. for $C_{18}H_{35}O_2$, 283.2637. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.58 (2H, m, H-2), 1.25 (30H, m, 15 × CH_2), 0.88 (3H, m, H-18). NMR and MS data comparison of compound 5 with those reported in the literature,[14] together with the comparison between compound 5 and standard in three different developing solvents (cyclohexane: EtOAc, 8:1; cyclohexane: acetone, 8:1; $CHCl_3$:EtOAc, 20:1) confirmed that compound 5 was octadecanoic acid.

Mixture of compounds 6 and 7: White amorphous powder. NMR spectra data for compound 6: ^{1}H NMR (400 MHz, DMSO-*d*6): δ_H 5.12 (1H, m, H-12), 2.99 (1H, m, H-3), 1.23, 1.03, 0.91, 0.89, 0.86, 0.74, 0.67 (each 3H, s, 7 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ_C 180.6 (C-28), 143.8 (C-13), 122.2 (C-12), 78.6 (C-3), 55.2 (C-5), 47.5 (C-9), 46.3 (C-17), 45.9 (C-19), 41.2 (C-14), 39.4 (C-18), 39.1 (C-8), 38.6 (C-1), 38.6 (C-4), 36.9 (C-10), 33.8 (C-21), 33.0 (C-29), 32.6 (C-7), 32.5 (C-22), 30.5 (C-20), 27.9 (C-23), 27.8 (C-15), 27.6 (C-2), 26.6 (C-27), 23.4 (C-30), 23.3 (C-16), 22.9 (C-11), 18.2 (C-6), 16.8 (C-26), 15.4 (C-24), 15.2 (C-25). NMR spectra data for compound **7:** ^{1}H NMR (400 MHz, DMSO-*d*6): δ_H 5.12 (1H, m, H-12), 2.99 (1H, m, H-3), 1.03, 0.89, 0.86, 0.74, 0.67 (each 3H, s, 5 × CH_3), 0.90 (3H, d, *J* = 7.0 Hz, CH_3), 0.81 (3H, d, *J* = 6.4 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ_C 180.6 (C-28), 138.1 (C-13), 125.4 (C-12), 78.6 (C-3), 55.2 (C-5), 52.8 (C-18), 47.7 (C-9), 47.5 (C-17), 41.2 (C-14), 39.4 (C-8), 39.1 (C-4), 39.0 (C-19), 38.6 (C-1), 38.4 (C-20), 36.9 (C-10), 36.7 (C-22), 33.0 (C-7), 30.5 (C-21), 27.9 (C-2), 27.6 (C-15), 27.6 (C-23), 23.2 (C-27), 22.9 (C-11), 20.9 (C-30), 18.2 (C-6), 16.8 (C-16), 16.7 (C-26), 16.6 (C-29), 15.4 (C-24), 15.1 (C-25). The mixture of compounds 6 and 7 was compared with standard samples (oleanolic acid and ursolic acid) by special TLC method, together with the comparison of NMR data with those reported in literature.[7] The comparison revealed that the mixture contained oleanolic acid and ursolic acid.

Compound 8: Yellow amorphous powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 9.65 (1H, d, *J* = 7.7 Hz, H-9), 7.40 (1H, d, *J* = 15.8 Hz, H-7), 7.12 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 7.07 (1H, d, *J* = 1.8 Hz, H-2), 6.96 (1H, d, *J* = 8.2 Hz, H-5), 6.59 (1H, dd, *J* = 7.7, 15.8 Hz, H-8), 3.95 (3H, s, 2-OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 193.6 (C-9), 153.1 (C-7), 149.0 (C-4), 147.0 (C-3), 126.6 (C-1), 126.4 (C-8), 124.0 (C-6), 114.9 (C-5), 109.5 (C-2), 56.0 (2-OMe). By comparing the NMR data of compound 8 with those reported in the literature,[15] it was identified as coniferaldehyde.

Compound 9: White amorphous powder. Melting point 144–146°C. ^{1}H NMR (400 MHz, CD_3OD): δ_H 7.53 (1H, d, *J* = 15.9 Hz, H-7), 7.03 (1H, d, *J* = 2.0 Hz, H-2), 6.93 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 6.77 (1H, d, *J* = 8.1 Hz, H-5), 6.24 (1H, d, *J* = 15.8 Hz, H-8), 4.21 (2H, q, *J* = 7.0 Hz, H-1'), 1.30 (3H, t, *J* = 7.1 Hz, H-2'). NMR data comparison of compound 9 with those reported in the literature,[16] in addition to the comparison between compound 9 and standards in three different developing solvents (cyclohexane: acetone, 2:1; cyclohexane: EtOAc, 1:1; $CHCl_3$:acetone, 4:1), confirmed that compound 9 was ethyl caffeate.

Compound 10: White amorphous powder. HR-ESI-MS (positive) *m/z* 432.3240 [M + Na] +, cald. for $C_{27}H_{44}O_4Na$, 432.3241. ^{1}H NMR (400 MHz, CD_3OD): δ_H 7.55 (1H, d, *J* = 15.7 Hz, H-7), 7.05 (1H, d, *J* = 2.0 Hz, H-2), 6.94 (1H, dd, *J* = 8.2, 2.0 Hz, H-6), 6.81 (1H, d, *J* = 8.1 Hz, H-5), 6.24 (1H, d, *J* = 15.8 Hz, H-8), 4.17 (2H, t, *J* = 6.6 Hz, H-1'), 1.71 (2H, m, H-2'), 0.88 (3H, t, *J* = 6.4 Hz, H-18'); ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ_C 168.9 (C-9), 148.4 (C-3), 146.1 (C-4), 145.7 (C-7), 127.0 (C-1), 122.3 (C-6), 115.8 (C-8), 114.8 (C-5), 114.5 (C-2), 65.1 (C-1'), 29.1 (C-2'), 26.4–30.1 (C-3' to C-15'), 32.3 (C-16'), 23.1 (C-17'), 14.2 (C-18'). By comparing the NMR and MS data of compound 10 with those reported in the literature,[17] it was identified as octadecanoyl caffeate.

Compound 11: Colorless needle (MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.50 (1H, d, *J* = 8.4 Hz, H-7), 6.24 (1H, d, *J* = 8.4 Hz, H-6), 5.22 (1H, dd, *J* = 15.2, 7.4 Hz, H-23), 5.13 (1H, dd, *J* = 15.2, 8.1 Hz, H-22), 3.97 (1H, m, H-3), 0.99 (3H, d, *J* = 6.6 Hz, H-21), 0.90 (3H, t, *J* = 6.8 Hz, H-28), 0.88 (3H, s, H-19), 0.83 (3H, d, *J* = 6.6 Hz, H-27), 0.81 (3H, s, H-18), 0.81 (3H, d, *J* = 6.6 Hz, H-26); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 135.4 (C-6), 135.2 (C-22), 132.3 (C-23), 130.7 (C-7), 82.1 (C-5), 79.4 (C-8), 66.4 (C-3), 56.1 (C-17), 51.6 (C-14), 51.0 (C-9), 44.5 (C-13), 42.7 (C-24), 39.7 (C-20), 39.3 (C-12), 36.9 (C-4), 36.9 (C-10), 34.6 (C-1), 33.0 (C-25), 30.1 (C-2), 28.6 (C-16), 23.4 (C-11), 20.8 (C-21), 20.6 (C-15), 19.9 (C-27), 19.6 (C-26), 18.1 (C-19), 17.5 (C-28), 12.8 (C-18). By comparing the NMR data of compound 11 with those reported in the literature,[18] it was identified as 5α,8α– peroxyergosterol.

Compound 12: Yellow amorphous powder. ^{1}H NMR (600 MHz, $CDCl_3$): δ_H 9.66 (1H, d, *J* = 7.6 Hz, H-9), 7.38 (1H, d, *J* = 15.7 Hz, H-7), 6.81 (2H, s, H-2 and H-6), 6.61 (1H, dd, *J* = 7.6, 15.7 Hz, H-8), 3.94 (6H, s, 2-OMe and 6-OMe); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 193.5 (C-9), 153.2 (C-7),

147.3 (C-3 and C-5), 138.0 (C-4), 126.7 (C-8), 125.5 (C-1), 105.5 (C-2 and C-6), 56.4 (2-OMe and 6-OMe). By comparing the NMR data of compound 12 with those reported in the literature,[19] it was identified as sinapaldehyde.

Compound 13: White amorphous powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.59 (1H, m, H-6), 3.85 (1H, m, H-7), 3.57 (1H, m, H-3) 1.00 (3H, s, H-19), 0.92 (3H, d, J = 6.4 Hz, H-21), 0.84 (3H, t, J = 7.6 Hz, H-29), 0.83 (3H, d, J = 7.6 Hz, H-26), 0.81 (3H, d, J = 7.0 Hz, H-27), 0.68 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 146.2 (C-5), 123.8 (C-6), 71.3 (C-3), 65.3 (C-7), 55.7 (C-17), 49.4 (C-14), 45.8 (C-24), 42.2 (C-9), 42.1 (C-13), 42.0 (C-4), 39.1 (C-12), 37.5 (C-8), 37.4 (C-10), 37.0 (C-1), 36.1 (C-20), 33.9 (C-22), 31.3 (C-2), 29.1 (C-25), 28.2 (C-16), 25.9 (C-15), 24.3 (C-23), 23.0 (C-28), 20.7 (C-11), 19.8 (C-26), 19.0 (C-27), 18.8 (C-21), 18.2 (C-19), 12.0 (C-29), 11.6 (C-18). By comparing the NMR data of compound 13 with those reported in the literature,[20] it was identified as 7 α-hydroxysitosterol.

DISCUSSION AND CONCLUSION

In Tibetan medicine, *Saussurea kingie*, *Saussurea sungpanensis*, and *Saussurea katochaete* are used as "གོན་པ་གས་སྐྱེས།" (transliteration: "gongbagaji") to stanch bleeding and treat furuncle.[2] *S. pachyneura* was also used as "གོན་པ་གས་སྐྱེས།" in some Tibetan areas.[21] This is the first report on the phytochemical research of *S. pachyneura*. In this study, we identified the following 13 compounds from the ethanolic extract of the whole plant material of *S. pachyneura*: four phenylpropanoids (compounds 8–10 and 12), three long-chain fatty acids/esters (compounds 1, 4, and 5), three steroids (compounds 3, 11, and 13), two triterpenoids (compounds 6 and 7), and one enyne (compound 2).

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Shi Z, Jin S. Delectis florae reipublicae popularis sinicae agenda academiae sinicae edita. Flora Republicae Popularis Sinicae. Vol. 18. Beijing: Science Press; 1999. p. 143-5.
2. Yang Y. Northwest institute of plateau biology, Chinese Academy of Sciences. Tibetan Medicine Records. Qinghai: Qinghai People's Publishing House; 1991.
3. Xie G, Tian J, Kövér KE, Mándi A, Kurtán T. Structural and stereochemical studies of a tetralin norsesquiterpenoid from *Ligularia kangtingensis*. Chirality 2014;26:574-9.
4. Tian J, Xie GB, Xie Y, Li TN. Terpenoids from *Ligularia kangtingensis*. Pharmacogn Mag 2015;11:44-7.
5. Xie GB, Xie Y, Hu YZ, Zhu ZX. Cytotoxic sesquiterpenoids from *Ligularia pleurocaulis*. Phytochemistry 2016;125:99-105.
6. Xie G, Wang X, Kurtán T, Mándi A, Wang T. Sibiralactone: A new monoterpene from *Sibiraea angustata*. Nat Prod Commun 2011;6:1799-800.
7. Hu YZ, Zhang WC, Zhang S, Xie GB. Secondary metabolites from Sibiraea angustata. Phcog Mag 2018;14:525-7.
8. Dong M, Oda Y, Hirota M. (10E,12Z,15Z)-9-hydroxy-10,12,15-octadecatrienoic acid methyl ester as an anti-inflammatory compound from *Ehretia dicksonii*. Biosci Biotechnol Biochem 2000;64:882-6.
9. Yadav JS, Deshpande PK, Sharma GV. Stereoselective synthesis of (S)-13-hydroxy octadeca-(9Z,11E)-di- and (9Z,11E,15Z)-trienoic acids: Selfdefensive substances against rice blast disease. Tetrahedron Lett 1992;48:4465-74.
10. Johnson DV, Griengl H. The chemoenzymatic synthesis of (S)-13-hydroxyoctadeca-(9Z,11E)-dienoic acid using the hydroxynitrile lyase from *Hevea brasiliensis*. Tetrahedron 1997;53:617-24.
11. Nakano H, Cantrell CL, Mamonov LK, Osbrink WL, Ross SA. Echinopsacetylenes A and B, new thiophenes from *Echinops transiliensis*. Org Lett 2011;13:6228-31.
12. Saito Y, Iwamoto Y, Okamoto Y, Gong X, Kuroda C, Tori M. Four new guaianolides and acetylenic alcohol from *Saussurea katochaete* collected in China. Nat Prod Commun 2012;7:447-50.
13. Gan L, Wang B, Liang H, Zhao YY, Jiang FC. Chemcial constituents from *Rubus alceaefolius Poir*. J Beijing Med Univ 2000;32:226-8.
14. Chen HG, Li M, Gong XJ, Zhou X, Tian Y. Chemical constituents from *Sammosilene tunicoides*. Chin Tradit Herbal Drugs 2010;41:204-6.
15. Sy LK, Brown GD. Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. Phytochemistry 1999;50:781-5.
16. Lee SJ, Jang HJ, Kim Y, Oh HM, Lee S, Jung K, *et al*. Inhibitory effects of Il-6-induced STAT3 activation of bio-active compounds derived from Salvia plebeian R.Br. Process Biochem 2016;51:2222-9.
17. García-Argáez AN, Pérez-Amador MC, Aguirre-Hernández E, Martínez-Vázquez M. Two new caffeate esters from roots of *Merremia tuberosa* and *M. dissecta*. Planta Med 1999;65:678-9.
18. Hybelbauerová S, Sejbal J, Dracínský M, Hahnová A, Koutek B. Chemical constituents of *Stereum subtomentosum* and two other birch-associated basidiomycetes: An interspecies comparative study. Chem Biodivers 2008;5:743-50.
19. Chen YQ, Su J, Shen YH, Zhang W, Hu XJ, Xu WZ, *et al*. Studies on chemical constituents of *Daphne holosericea* (Diels) Hamay a. Chin Pharm J 2008;43:1453-6.
20. Liu YF, Yang XW, Wu B. Chemical constituents of the flower buds of *Tussilago farfara*. J Chin Pharm Sci 2007;16:288-93.
21. Gong HD, Xie DF. Investigation of the traditional Tibetan medicine plant resource of the Saussurea DC. In the east of Qinghai-Tibet plateau. J Anhui Agri Sci 2009;37:171-2.