SUPPLEMENTARY MATERIAL

A new sesquiterpenoid from *Saussurea lappa* roots

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A new sesquiterpenoid from *Saussurea lappa* roots

A new sesquiterpenoid, 10 α -methoxyartemisinic acid (**1**) was isolated from *Saussurea lappa* roots along with two known compound, costunolide (**2**) and dehydrocostus lactone (**3**) by high-speed counter-current chromatography (HSCCC) with purities of 85%, 95% and 98%, respectively. Their structures were determined on the basis of spectral data including 1D and 2D NMR and HREIMS.

Keywords: *Saussurea lappa* Roots; a new sesquiterpene; 10-methoxy-artemisinic acid; HSCCC;

Contents:

Experimental

S1 Apparatus

S2 Plant material and Reagents

S3 Extraction and isolation

S4 Analysis of preparation product

References

Table S1. NMR spectroscopic data for compound **1** (CDCl₃, 600 MHz)

Figure S1. The HSCCC chromatogram of petroleum ether-soluble fraction of *Saussurea lappa* Root (petroleum ether-ethyl acetate-methanol-water (5:1:6.5:3.5), 2.5mL/min, 800rpm. 1: 10-methoxy-artemisinic acid, 2: costunolide, 3: dehydrocostus lactone; detection wavelength: 254nm; sample injection: 20mL.)

Figure S2. HPLC analyses and structures of the fractions obtained from *Saussurea lappa* roots by HSCCC. A: **1**. 10-methoxy-artemisinic acid; B: **2**. costunolide; C: **3**. dehydrocostus lactone.

Figure S3. HPLC analysis of 10-methoxy-artemisinic acid

Figure S4. ¹³C NMR spectrum (600MHz) of compound **1**

Figure S5. ¹H NMR spectrum (600MHz) of compound **1**

Figure S6. Key ¹H-¹H COSY (—) and HMBC (→) correlations of compound **1**

Figure S7. HSQC spectrum (600MHz) of compound **1**

Figure S8. ¹H-¹H COSY spectrum (600MHz) of compound **1**

Figure S9. HMBC spectrum (600MHz) of compound **1**

Figure S10. The possible biosynthetic pathway (C) of compound **1**

Experimental

Apparatus

Separation was performed by the OptiChrome™-A double-action column semi-preparation high-speed counter-current chromatograph (CounterCurrent Technology Co.,Ltd., Jiangsu, China); The instrument was a fully automated system consisting of a 2 polytetrafluoroethylene (PTFE) preparative coils (i.d of tube, 1.59mm; revolution radius, 80cm; range of β , 0.50-0.80; total volume, 360mL) and a 20-mL sample loop. The HSCCC system was equipped with a model UV-3000 detector (Beijing Chuangxin Tongheng Science and Technology Co., Ltd), a model CXTH3000 workstation. The spots were observed under UV light and visualized by spraying with 10% aq; H₂SO₄ solution followed by heating at 120 for 5 min. HRESIMS was carried out in the positive ion mode on a Waters ACQUITY SQD UPLC system coupled to a Micromass Q-ToF Micro mass spectrometer. NMR spectra were obtained on an Agilent 600 MHz DD2 nuclear magnetic resonance (NMR) spectrometer (Agilent Technologies Inc., USA); The partition coefficients were measured on an Agilent 1260 HPLC system (Agilent Technologies Inc., USA).

Plant material and Reagents

All chemicals including organic solvents used for sample preparation and HSCCC were of analytical grade and purchased from Chuandong Chemical Factory (Chongqing, China). Methanol and acetonitrile used for HPLC analysis was of chromatographic grade (Adamas-beta Chemical reagent Co., Ltd. Switzerland), and water was distilled.

Saussurea lappa roots were collected in Kaixian, Chongqing, China in 2012 and identified by Dr Junjiang Lv, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (NO. 2012005) was deposited at the herbarium of the department of pharmacy, University of Chongqing, Chongqing.

Extraction and isolation

Two kilograms dried roots of *S. lappa* were cut into chunks and extracted with 5L water at 85°C for 3h (3×), and the combined aqueous extract was evaporated at 65°C under reduced pressure. The extracts were subsequently dissolved in water, partitioned with equal volume petroleum ether (PE) (60-90°C, 3×). Solvent was evaporated and the residue was freeze-dried to afford the PE fraction (25.3 g), which was subjected to

subsequent HSCCC separation and purification.

The preparative HSCCC was applied for isolation and the process was performed as follows: The system solvent petroleum ether-ethyl acetate-methanol-water (5:1:6.5:3.5) was prepared by mixing the solvents in a separation funnel according to the volume ratios and thoroughly equilibrated by shaking them vigorously. Then, they were left overnight and two phases were separated and degassed by sonication for 30 min prior to use. The multilayer coil column was first entirely filled with upper phase as a stationary phase at the flow rate of 20 mL/min. The lower aqueous mobile phase was then pumped into the head end of the column inlet at a flow-rate of 3 mL/min, while the apparatus was rotated at 800 rpm at room temperature (25 °C). When the hydrodynamic equilibrium was established, 500mg of crude extract was dissolved in 20 mL mixture solution of lower phase and upper phase of (1:1, v/v) was injected through the sample loop. The eluent was collected in glass tubes with a fraction collector, and peak fractions were concentrated and dried after combining similar fractions based on the UV chromatogram recorded at 254 nm in the monitor. After the separation was completed, the solvents in the column were pushed out and the retention of stationary phase was measured. Peak resolution was computed according to the chromatogram. The peak fractions were purified further and the purified compounds were stored at -20°C before HPLC and ¹H and ¹³C NMR analyses.

Analysis of preparation product

HPLC analyses of the crude sample and the HSCCC peak fractions were performed with a Kromasil C₁₈ column (250 × 4.6mm, 5μm) at 25 °C. Methanol- water (85: 15, v/v) was used as the mobile phase. The flow rate was 0.8 mL/min and the column effluent was monitored by use of the DAD at 254 nm, and the injection volume of sample was 10 μL. Identification of each isolated target compound was carried out by ¹H- NMR, ¹³C- NMR, 1H-1H Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Correlation Spectroscopy (HSQC), Heteronuclear Multiple Quantum Correlation Spectroscopy (HMQC). The structure of dehydrocostus lactone and costunolide was identified by ¹H and ¹³C NMR.

The chemical structure identification of the three compounds was carried out by MS, ¹H-NMR, ¹³C-NMR spectra as followers. Compound **1** was obtained as white amorphous powder, with a molecular formula C₁₆H₂₄O₃ and a unsaturation of 5,

deduced from HRESIMS m/z 265.181 $[M+H]^+$ (calcd for $C_{16}H_{25}O_3$ 265.180). The 1H , ^{13}C NMR and HSQC data of the new sesquiterpene (peak **1**) were shown in Table S1. Moreover, the 1H - 1H COSY and HMBC spectrum were shown in the Figure S4-S5. Based on all these considerations, peak **1** was assigned as 10-methoxy-artemisinic acid, which is a new sesquiterpene. In this paper, a possible biosynthetic pathway of 10-methoxy-artemisinic acid was proposed that 10-methoxy-artemisinic acid might be synthesized from the bioprecursor Farnesyl Pyrophosphate (FPP), rather than from dehydrocostus lactone, as shown in the Figure S10. (Duan JA et al. 2010, Kraker JW et al. 2001).

Peak **2**. 1H NMR (500 MHz, $CDCl_3$), δ (ppm): 6.25 (1H, d, $J=3.5$ Hz, H-13a), 5.51 (1H, d, $J=3.0$ Hz, H-13b), 4.85 (1H, dd, $J=11.0, 4.0$ Hz, H-1), 4.73 (1H, d, $J=10.0$ Hz, H-5), 4.57 (1H, t, $J=9.5$ Hz, H-6), 1.68 (3H, s, H-15), 1.41 (3H, s, H-14). ^{13}C NMR (500 MHz, $CDCl_3$), δ (ppm): 170.59 (C-12), 141.60 (C-11), 140.23 (C-4), 137.10 (C-10), 127.42 (C-5), 127.18 (C-1), 119.77 (C-13), 82.04 (C-6), 50.55 (C-7), 41.12 (C-3), 39.60 (C-9), 28.18 (C-2), 26.33 (C-8), 17.48 (C-15), 16.24 (C-14). Comparing the data with the literature (Dhilion RS et al. 1987), peak **2** was identified as costunolide.

Peak **3**. 1H NMR (500 MHz, $CDCl_3$), δ (ppm): 6.18 (1H, d, $J=3.5$ Hz, H-13a), 5.45 (1H, d, $J=3.0$ Hz, H-13b), 5.24 (1H, brs, H-15a), 5.03 (1H, brs, H-15b), 4.87 (1H, brs, H-14a), 4.78 (1H, brs, H-14b), 3.95 (1H, t, $J=9.5$ Hz, H-5). ^{13}C NMR (500 MHz, $CDCl_3$), δ (ppm): 170.33 (C-12), 151.39 (C-11), 149.35 (C-3), 139.87 (C-9), 120.26 (C-13), 112.70 (C-15), 109.65 (C-14), 85.35 (C-5), 52.13 (C-4), 47.70 (C-6), 45.22 (C-10), 36.39 (C-2), 32.72 (C-8), 31.05 (C-7), 30.41 (C-1). Comparing the data with the literature (Dhilion RS et al. 1987), peak **3** was identified as dehydrocostus lactone.

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Table S1. NMR spectroscopic data for compound **1** (CDCl₃, 600 MHz)

No.	δ C	δ H
1	40.46	1.95(1H, m)
2	21.62	1.99(1H, m); 1.59(1H, m)
3	28.91	1.96(1H, m); 1.91(1H, m)
4	135.54	
5	121.73	5.16(1H, brs)
6	35.63	2.78(1H, m)
7	40.20	2.87(1H, m)
8	24.06	1.67(1H, m); 1.51(1H, m)
9	34.49	1.76(1H, m); 1.60(1H, m)
10	76.73	
11	143.73	
12	172.92	
13	125.25	6.32(1H, s); 5.31(1H, s)
14	21.16	1.19(3H, s)
15	23.44	1.56(3H, s)
16	47.93	3.20(3H, s)

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Figure S7. HSQC spectrum (600MHz) of compound **1**

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