A new sesquiterpenoid from *Saussurea lappa* roots

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1. Introduction

Muxiang is the roots of *Saussurea lappa* C.B Clarke, which has been widely used in India, China and Japan for the treatment of asthma, cough, diarrhoea, vomit, indigestion, colic, cholecystitis, cancer and hepatitis (Cho et al. 2000). Sesquiterpenes lactones, such as costunolide and dehydrocostus lactone, were representative active compounds of *S. lappa* roots. It displayed multiple biological activities such as anticancer, anti-inflammatory, antiulcer, hepatoprotective and immunomodulatory activities (Li et al. 2005). Moreover, they are also the major index compounds for the quality control of *S. lappa* roots.

As a part of ongoing study to find new active compounds from *S. lappa* roots, a new compound (1) was isolated along with two known compounds (2–3) by high-speed countercurrent chromatography (HSCCC). HSCCC as a unique form of free liquid-liquid partition chromatography has been widely used in the separation and purification of natural products with low risk of sample denaturation and irreversible adsorption, high sample recovery and large loading capacity. (Liu et al. 2014; Tuba et al. 2014; Zhang et al. 2015). In this study, a ternary solvent system was applied to fast isolate and purify...
dehydrocostus lactone, costunolide and a new sesquiterpene named 10-methoxy-artemisinic acid which might be used for further chemical research and pharmacological studies or as reference substances by preparative HSCCC (Figure 1). Their chemical structures were elucidated by 1D and 2D NMR and HRMS experiments. The results were discussed herein.

2. Results and discussion

Under the obtained preparative HSCCC conditions, three sesquiterpenoid lactones were isolated and purified from petroleum ether (PE) extract of *S. lappa* roots (Figure S1). The purity of compounds 1, 2 and 3 was 85, 98 and 95%, respectively, as determined by HPLC (Figure S2). And then, a silica gel column (1.5 cm × 15 cm) was used to purify further the new compound (Xu et al. 2009). Petroleum ether–ethyl acetate system was used as elution system, and the higher purity 10-methoxy-artemisinic was obtained with petroleum ether–ethyl acetate (15:1). According to HPLC detection (Figure S3), the purity of the new sesquiterpenoid after a small purification step was 97.8%. The amount of the three separated products in one-step preparation process could reach 10–20, 140–150 and 150–180 mg, respectively. Two known compounds were identified as costunolide (2) and dehydrocostus lactone (3) (Figure 1, Dhillon et al. 1987).

2.1 Structure elucidation of new compound

Compound 1 was obtained as a white amorphous powder, with a molecular formula C_{16}H_{24}O_{3} and an unsaturation of five, deduced from HRESIMS m/z 265.181 [M + H]+ (calcd for C_{16}H_{25}O_{3} 265.180). 13C NMR and DEPT spectra displayed 16 carbon signals (Table S1, Figure S4), including one carboxyl group at \( \delta_{c} 172.9 \), three methyl groups (one methoxyl at \( \delta_{c} 47.9 \)), five methylenes (one terminal double bond at \( \delta_{c} 125.3 \)), four methines (one olefinic methine at \( \delta_{c} 121.7 \)) and three quaternary carbons (two sp2 carbons). In the \(^1\)H NMR spectrum (Figure S5), three olefinic protons at \( \delta_{H} 6.32(1H, \text{ brs}) \), 5.31(1H, brs) and 5.16(1H, brs), two methyl groups at \( \delta_{H} 1.19(3H, \text{ s}) \) and 1.56(3H, s) and one methoxyl at 3.20 (3H, s), were observed. Besides two olefinic bonds and one carboxyl group, there were two rings in the structure of 1, taking

Figure 1. Structures of compounds 1–3 from petroleum ether-soluble fraction of *S. lappa* root.
into account the unsaturation of 5. Compound 1 was a sesquiterpenoid with two rings. The above-mentioned NMR characteristics were close to those of 10α-hydroxyartemisinic acid. The $^{13}$C NMR data of compound 1 were comparable to those of 10α-hydroxyartemisinic acid, except for C-1 ($\delta_c$ 40.5) and C-9 ($\delta_c$ 34.5), of which the chemical shifts were up-field shifted to 5.5 and 8.6 ppm. These differences were attributable to the methoxyl group substitution at C-10 in compound 1, while it was a hydroxyl group at C-10 in 10α-hydroxyartemisinic acid. The connectivity of the methyl group and C-10 was confirmed by the HMBC correlation from the signals of methoxyl protons [$\delta_H$ 3.20 (3H, s)] to C-10 (Figure S6). Together with the $^1$H–$^1$H COSY, ROESY, HSQC and HMBC spectra (Figure S6–S9), the planar structure of 1 was elucidated as in Figure S6.

The relative configuration of 1 was constructed by proton coupling constants analysis and ROESY experiments. The small coupling constants $J_{H-1,H-6} = 4.5$, $J_{H-7,H-6} = 4.5$ Hz suggested that H-1, H-6 and H-7 were at the same side. The ROESY correlation of H-7 and the methoxyl group implied $\alpha$ orientation of methoxyl group. Thus, the structure of compound 1 was elucidated and named as 10α-methoxyartemisinic acid (Figure 1).

3. Conclusions

In order to fast isolate and prepare new compounds from $S$. lappa roots, we used HSCCC and the spectroscopy techniques of NMR, MS and UV, to isolate and identify three sesquiterpenoid lactones from $S$. lappa roots (Ren et al. 2007). In this paper, a ternary solvent system petroleum ether–ethyl acetate–methanol–water (5:1:6.5:3.5, v/v/v/v) was applied to isolate and prepare active compounds by preparative HSCCC. As a result, one new sesquiterpenoid 10-methoxy-artemisinic acid (1) was isolated from $S$. lappa roots, along with two known ones costunolide (2) and dehydrocostus lactone (3) with purities of 85, 95 and 98%, respectively.

Supplementary material

Experimental details relating to this article are available online, alongside Tables S1 and Figures S1–S10.

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