Secondary metabolites from *Ligularia sagitta*

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1. **Subject and source**

*Ligularia sagitta* (Maxim.) Mattf. (Asteraceae) is widely distributed in Northwestern China, its roots and rhizomes have long been used as folk medicine to reduce phlegm, to relieve cough, and for treatment of pulmonary tuberculosis, urinary track blockages, common cold, pharyngitis as well (Jiangsu College of New Medicine, 1977). The plant material of *L. sagitta* was collected in Datong city (E 101°49′17″, N 36°34′3″, alt. 2300—2400 m), Qinghai province of China in August 2003, and authenticated by Prof. Shang-Wu Liu, Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining, China. A voucher specimen for this material (LS057) was deposited in the herbarium of China Pharmaceutical University (CPU).

2. **Previous work**

Monoterpenes (Li et al., 2003), sesquiterpenes (Zhao et al., 1994; Liu et al., 1995; Yang et al., 1995; Peng et al., 1997; Li et al., 2003), diterpenes (Chen et al., 1992a,b), triterpenes (Li et al., 2003), β-sitosterol and daucosterol have been previously reported from this plant. In addition, the antibacterial activity of some sesquiterpenes of *L. sagitta* has been carried out (Li et al., 2003).

3. **Present study**

The air-dried and powdered roots and rhizomes of *L. sagitta* (4.5 kg) were extracted under reflux with 90% EtOH (10 l × 3). The concentrated extract (570 g) obtained under reduced pressure was suspended in H2O containing 0.2%
H$_2$SO$_4$ and filtered. The insoluble residue was washed with H$_2$O to neutral (pH 7), then partitioned with EtOAc (21 × 3) to give 370 g of EtOAc extract.

The EtOAc extract was subjected to silica gel column (200–300 mesh) chromatography eluted with petroleum ether (60–90 °C) and gradually increasing the polarity of the solvent by adding EtOAc. According to the TLC analyses, 10 crude fractions (Frs. 1–10) were obtained. Benzofuranorhemophil-1-ene (1, 40 mg) (Chen et al., 1992a,b; Liu et al., 1995) and bakkenolide A (2, 7 g) (Chen et al., 1992a,b) were subsequently crystallized, respectively, from Frs. 1 and 2. Fr. 3 was subjected to silica gel column eluted with petrol ether–EtOAc in gradient to yield 7-hydroxy-9(10)-ene-1,8-diox-6,7-dihydrofuranoeremophilane (3, 9 mg) (Jia et al., 1992), lupeol (4, 25 mg) (Li et al., 2003) and β-sitosterol (5, 90 mg) (Zhao et al., 1994). Fr. 4 was subjected to silica gel column eluted with a gradient of petroleum ether–EtOAc, and further purified by Sephadex LH-20 with CH$_3$Cl–MeOH (1:1) to give ferulic acid (6, 20 mg) (Charies, 1976), p-coumaric acid (7, 10 mg) (Cheng et al., 2002). Fr. 5 was subjected to silica gel column eluted with petrol ether–EtOAc in gradient, and further purified by Sephadex LH-20 with CH$_3$Cl–MeOH (1:1) to give caffeic acid (8, 20 mg) (Zhang et al., 2005). Fr. 8 was subjected to silica gel column eluted with CH$_3$Cl–MeOH in gradient to yield daucosterol (9, 30 mg) (Zhao et al., 1994) and 3,5-di-O-caffeoylquinic acid (10, 25 mg) (Kodoma et al., 1998). These compounds were identified by means of spectroscopic methods (MS, $^1$H NMR, $^{13}$C NMR), and confirmed by comparison with the literature data.

4. Chemotaxonomic significance

*Ligularia* Cass. belongs to the tribe Senecioneae of the family Asteraceae, comprising about 130 species classified into six sections and 11 series based on their morphologies as the shape of lamina, leaf venation (palmate veins or pinnate veins), and arranging of capitula (in corymb-like or raceme-like) (Liu et al., 1994). *L. sagitta* belongs to the series *Ligularia* in section *Ligularia*. The characteristic secondary metabolites of the genus *Ligularia* are eremophilane sesquiterpenes and pyrrolizidine alkaloids, the latter are hepatotoxic to livestock and human being. Nevertheless, parts of the species like *L. sagitta* contain only eremophilane sesquiterpenes as their main chemical components as reported previously, and no pyrrolizidine alkaloids were present.

Among the 10 secondary products isolated in this study, 3,5-di-O-caffeoylquinic acid (10) and p-coumaric acid (7) are reported for the first time from the genus *Ligularia*, although 10 occurs in the genus *Artemisa* and the genus *Lychnophora* of the family Asteraceae (Carnata et al., 2000; dos Santos et al., 2005) and also in other families, such as the family Rosaceae (Motoichiro et al., 1998) and the family Caprifoliaceae (Peng et al., 2000). Ferulic acid (6), caffeic acid (8), and 7-hydroxy-9(10)-ene-1,8-diox-6,7-dihydrofuranoeremophilane (3) were isolated for the first time from this species. Compound 3 is also present in *Ligularia veitchiana* (Jia et al., 1992) of the same section and can be consider as a useful taxonomic marker for this section. Bakkenolide A (2), a special sesquiterpene lactone, is rich in *L. sagitta* and widespread in the genus *Ligularia* (Alberto et al., 1991) as well as in the genus *Petasites* (George et al., 1976), suggesting that these two genera may related chemosystematically. In classical taxonomy, both the genus...
Sagitta and genus Petasites belongs to the same subtribe Tussilagininae based on their similar morphological characters of revolute spires, palmate or reniform leaves, palmate veins, white tomentose, and monolayer tepal as well. A phylogenetic tree of 52 species of the Senecioneae by using chloroplast (ndhF and trnL-F) and (ITS) sequences indicated that Tussilago, Petasites, Ligularia and Farfugium are similar in chloroplast and nuclear DNA sequences.

The existence of bakkenolide A in both these two genera can partly provide a chemotaxonomic evidence to support the morphological classification and molecular systematics’ result. Whether the other closed genera as Tussilago and Farfugium contain also Bakkenolide A should be elucidated in further phytochemical investigation.

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References