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Chemical and Genetic Diversity of Some *Ligularia* Species (Compositae) in Northwestern Yunnan and Southwestern Sichuan of China

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1 Introduction

Ligularia Cass., (Compositae) is a highly diversified genus, and more than 100 species of which are distributed in the eastern Qinghai-Tibet Plateau and adjacent areas. *Ligularia* species have been studied with respect to secondary metabolites, and many sesquiterpenes of the furanoeremophilane type have been isolated from them. In order to find correlates among these variations, and ultimately understand the diversity-generating mechanism of *Ligularia* species in the Hengduan Mountains, we initiated an extensive study that uses furanoeremophilanes as a chemical index and the DNA sequence as a genetic index. Furanoeremophilanes have been detected conventionally by Ehrlich's test, which has been used in a search for novel natural products. As for the DNA sequence, we determined the nucleotide sequence of the *atpB-rbcL* intergenic region in the present study.

2 Results and Discussion

Our first study was made on *L. tongolensis*, *L. cymbulifera*, both belonging to the section Corymbosae, and distribute widely from Lijiang area to Daocheng area (see map). Nineteen samples of *L. tongolensis* and 13 samples of *L. cymbulifera* were collected. Without drying, the plant materials were extracted with ethanol, and the extracted alcoholic solutions were subjected to Ehrlich's test on TLC. Furanoeremophilanes were found to be produced, and compounds **1-4** were isolated from *L. tongolensis*, and **3-6** from *L. cymbulifera*. Compounds **1** and **2** were found to be new, and their structure was determined from careful analysis of NMR. DNA was purified from leaves of the same samples and the nucleotide sequence of the *atpB-rbcL* region was determined. Differences among the variants were observed at the 28th nucleotide (A or G) and at an A stretch around the 510th base (9, 10, 11, or 12).

The intra-specific diversity of *L. tongolensis* and *L. cymbulifera* were in contrast. Four out of 19 *L. tongolensis* samples contained a strongly Ehrlich-positive compound besides a number of positive compounds, and five variants of the *atpB-rbcL* sequence were found in these samples. The presence of the strongly Ehrlich-positive compound and the *atpB-rbcL* sequence type did not coincide. With the two indexes, the 19 samples of *L. tongolensis* could be classified into 7 groups. In contrast, no variation was observed in 13 *L.*

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cymbulifera samples with respect to the furanoeremophilane composition or the *atpB-rbcL* sequence. The lack of chemical and genetic diversity in *L. cymbulifera* probably results from the uniformity of its habitat. *L. tongolensis* is probably adapting to various habitats, and is still in the process of differentiation.

L. vellerea, belonging to the section Scapicaulis, was also studied. Eighteen samples were collected, among which 10 samples, obtained near Zhongdian city, showed the same TLC and compound **7** was isolated as the major component. DNA sequence of these samples was also the same with few exceptions. Interestingly, chemical constituents of 3 samples collected near Luguhu were completely different, the structure of which is under investigation. See Fig. 1.

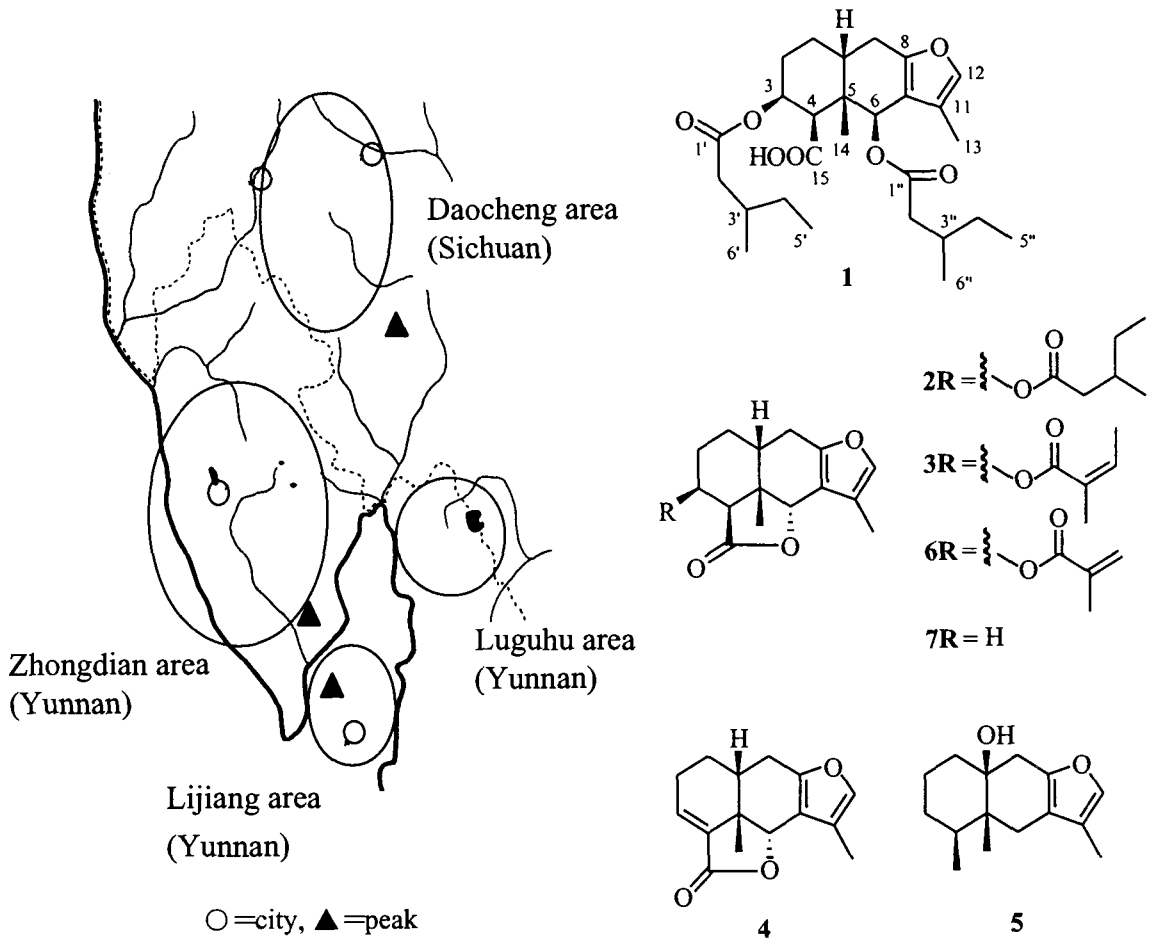

Fig. 1 Chemical and genetic diversity of some *Ligularia* species in Yunnan and Sichuan of China

Note: A part of this work has been published in *Bull. Chem. Soc. Japan*, 2005.