

Short communication

Intra-specific diversity of the chemical composition of *Ligularia lamarum* in the Hengduan Mountains, China: The structures of four new eremophilanes and a new seco-eremophilane

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ABSTRACT

Chemical compositions and internal transcribed spacer (ITS) sequences of five samples of *Ligularia lamarum* collected in Sichuan Province, China, were analyzed. Fourteen compounds, including four new eremophilanes and one new seco-eremophilane, were isolated and their structures were elucidated by spectroscopic methods. Intra-specific diversity in the chemical composition was found to be higher than previously known. The result of DNA analysis suggested that one of the samples was introgressed, although its chemical composition was typical of *L. lamarum*.

1. Introduction

Ligularia (Asteraceae) species in the Hengduan Mountains of China are highly diversified, thereby providing an opportunity for studying the diversity in secondary metabolites. We have studied the diversity of *Ligularia* using root chemicals and evolutionally neutral DNA sequences as indices, because the roots of *Ligularia* contain a variety of terpenoids. To date, we have revealed intra-specific diversity in many species with various modes. For example, many species consisted of several chemotypes (Kuroda et al., 2012, 2014), implying that the generation mechanism of chemical diversity is complex. Furanoeremophilanes have been isolated from most of the major species of *Ligularia*, and other compounds such as bisabolanes, benzofurans, and phenylpropenoids have also been obtained.

L. lamarum (Diels) C. C. Chang is widely distributed in the Hengduan Mountains, including the Yunnan, Sichuan, and Gansu provinces and the Xizang Autonomous Region of China and northwestern Myanmar (Liu and Illarionova, 2011). Taxonomically, the species is closely related to *L. subspicata* (Bureau and Franch.) Hand.-

Mazz. The major morphological difference between *L. lamarum* and *L. subspicata* is the presence (*L. lamarum*) or absence (*L. subspicata*) of ray florets (Liu and Illarionova, 2011). We previously analyzed *L. lamarum* and *L. subspicata* collected in northwestern Yunnan and southwestern Sichuan, and showed that the two species were indistinguishable by our two indices (Saito et al., 2011). Eremophilanes were isolated from all of our collected samples, which were classified into two chemotypes: 1) a furanoeremophilane type and 2) an eremophilan-8-one type. Subspicatin (1 β -acyloxyfuranoeremophilanes) are characteristic of the furanoeremophilane type, although not all furanoeremophilane-type samples produce subspicatin(s).

Here, we report the results of analyses of *L. lamarum* samples collected in northern Sichuan Province, which showed further diversity within the furanoeremophilane type. Five new compounds were isolated and their structures were determined.

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Table 1
Collection localities and chemical composition of *Ligularia lamarum* samples.

Sample no.	Specimen no.	Location ^a	Altitude (m)	Isolated compounds
1	2010–09	Zhegushan (Hongyuan)	3900	1, 4, 5, 13
2	2010–10	Zhegushan (Hongyuan)	3900	1, 7
3	2011–53	Queershan, east side (Dege)	4100	1, 2, 8, 9
4	2011–54	Queershan, east side (Dege)	4100	1, 2, 3, 6, 7
5	2011–56	Queershan, west side (Dege)	4400	1, 2, 6–8, 10–12, 14
6 ^b	2008–55	Laojunshan (Jianchuan)	4000	
7 ^b	2009–95	Maerkang/Xianjin (Maerkang)	3900	

^a County in parenthesis. Jianchuan County is situated within Yunnan Province, whereas the others are situated within Sichuan Province.

^b Previously analyzed sample (Saito et al., 2011). Samples 6 and 7 correspond to samples 7 and 9, respectively, in the report.

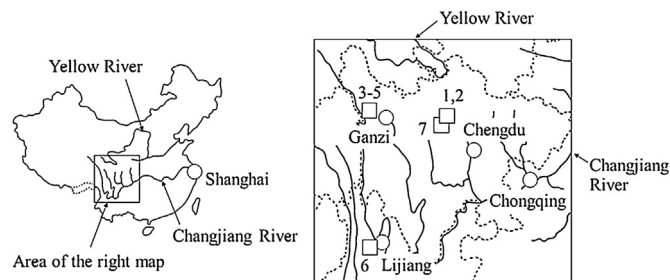


Fig. 1. Locations of the collected samples of *L. lamarum* (squares). Circles indicate major cities. Solid and dotted lines indicate rivers and boundaries of provinces, respectively.

2. Results

2.1. Samples

Five samples of *L. lamarum* were collected in northern Sichuan Province (Table 1 and Fig. 1): two in Hongyuan County (samples 1 and 2) and three in Dege County (samples 3–5). Samples 1 and 2 were less than 100 m apart. Sample 1 had typical ray florets, whereas sample 2 had very small ray florets. Samples 3 and 4 were collected sympatrically. The leaves of sample 3 were more cordate, whereas those of sample 4 were more sagittate. Sample 5 was collected approximately 10 km west of the locations of samples 3 and 4.

2.2. Chemical analysis

Compounds of the roots of each sample were extracted with EtOH immediately without drying and the extracts were subjected to Ehrlich's test on TLC plates. All five samples were Ehrlich-positive, suggesting the presence of furanoeremophilanes (Kuroda et al., 2004; Kuroda and Nishio, 2007). Although samples 1 and 2 were collected close to each other, they showed different TLC patterns. Sample 1 showed a blue spot at $R_f = 0.48$ (hexane/EtOAc 7:3), whereas sample 2 showed two purple spots: one at $R_f = 0.73$ and one at 0.39. Samples 3–5 showed the same spots as in sample 2, suggesting that the major chemical constituents were very similar.

The extracts were analyzed by LC–MS. The total ion chromatograms (TICs) are shown in Fig. 2. The chromatograms of samples 2–5 were almost identical, in agreement with the TLC results. The EtOH extracts of two previous samples (samples 6 and 7 (Saito et al., 2011; see Table 1)) were also subjected to LC–MS. The TICs of samples 2–5 were very similar to that of sample 7, but differed from that of sample 6.

Fourteen compounds, including eremophilane-type sesquiterpenes 1–12, α -bisabolol, and lupeol, were isolated from the dried roots (Fig. 3). From sample 1, 1 (Tori et al., 2008), 4, 5, and α -bisabolol were isolated (Table 1). From sample 2, 1 and 7 were isolated. From sample 3, 1, 2 (Nagano et al., 1982), 8 (Shimizu et al., 2014a), and 9 were isolated. From sample 4, 1, 2, 3 (Tada et al., 1971), 6 (ligularone; Ishii et al., 1965; Koike et al., 1999), and 7 were isolated. From sample 5, 1, 2, 6, 7, 8, 10 (Tori et al., 2008), 11 (Saito et al., 2015), 12, and

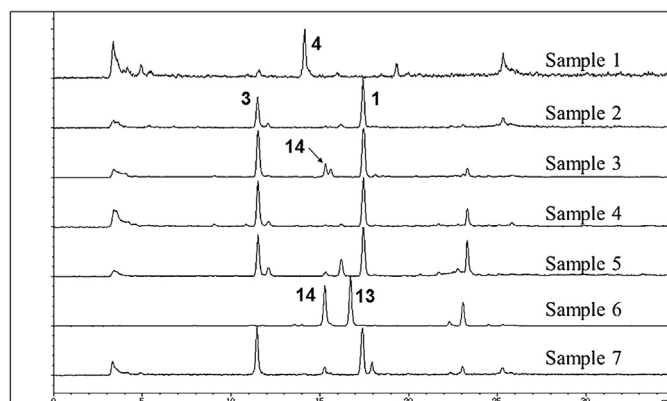


Fig. 2. LC profiles (total ion chromatograms) for samples 1–7. Mass spectra of 1, 3, 4, 13, and 14 were m/z 233 ($[M - OEt]^+$), 233 ($[M - OH]^+$), 329 ($[M - OH]^+$), 333 ($[M + H]^+$), and 235 ($[M + H]^+$), respectively.

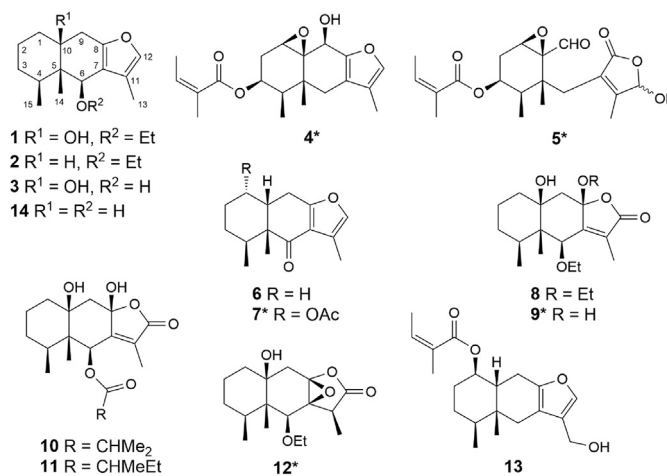


Fig. 3. Components of *L. lamarum* (asterisks indicate new compounds).

lupeol were isolated.

Of the 14 compounds, 4, 5, 7, 9, and 12 were new. Their structures were determined as detailed below.

The molecular formula of 4 was determined to be $C_{20}H_{26}O_5$ from high-resolution CI-MS (HRCIMS) (m/z 346.1773; M^+) and ^{13}C NMR data. The IR spectrum showed the presence of a hydroxy group (3510 cm^{-1}) and a conjugated ester carbonyl group (1712 cm^{-1}). The 1H and ^{13}C NMR spectra showed typical signals of furanoeremophilane [δ_H 7.20 (q, 1.1 Hz, H-12), 1.95 (d, 1.1 Hz, Me-13), 1.36 (s, Me-14), and 1.11 (d, 7.2 Hz, Me-15); δ_C 140.4 (C-12), 8.0 (C-13), 23.7 (C-14), and 9.8 (C-15)] as well as those of an angeloyl group [δ_H 6.10 (qq, 7.3, 1.4 Hz, H-3'), 2.00 (dq, 7.3, 1.4 Hz, Me-4'), and 1.89 (quint, 1.4 Hz, Me-5'); δ_C 167.4 (C-1'), 127.8 (C-2'), 138.4 (C-3'), 15.8 (C-4'), and 20.6 (C-5')] (Tables 2 and 3). Signals of three oxygenated methines were also observed [δ_H 3.29 (d, 5.6 Hz, H-1), 5.21 (ddd, 11.5, 7.1, 4.2 Hz, H-3),

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