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Chemical constituents of *Ligularia virgaurea* and its diversity in southwestern Sichuan of China

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Abstract—Chemical constituents of root extract and the nucleotide sequences of the *atpB*–*rbcL* intergenic region and the internal transcribed spacers (ITSs) of the ribosomal RNA gene were studied for *Ligularia virgaurea* var. *virgaurea* collected in southwestern Sichuan Province of China. Eleven samples were collected. Four of them were found to contain four new furanoeremophilanes, virgaurenones A–D, as well as a new eremophilanolide, virgaurenolide A. The other samples contained different furanoeremophilanes and their derivatives including nor-type of compounds, two of which were new. Diversity was found to be present in the nucleotide sequences as well. The chemical composition was found to be correlated with the ITS variation but not with the geographic distribution of the samples.
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1. Introduction

Ligularia Cass. (Compositae (or Asteraceae)) is a genus highly diversified in the eastern Qinghai–Tibet Plateau and adjacent areas, and more than 100 species are extant therein.¹ The genus is diversified particularly in the Hengduan Mountains and this area is considered to be the main center of the on-going evolution of the genus.² Therefore, *Ligularia* species in the Hengduan Mountains provide a good set of subjects for the study of plant evolution. We have been studying inter- and intra-specific diversity of *Ligularia* in the Hengduan Mountains by the combination of chemical and genetic approaches. As the chemical index, we have chosen furanoeremophilanes, since they have been found in *Ligularia*³ as well as related genera such as *Farfugium*,⁴ *Petasites*,⁵ and *Syneilesis*,⁶ and since the presence/absence of furanosesquiterpenes can be easily examined by Ehrlich's test on TLC.⁷ As the genetic index, we have chosen the DNA sequences of the *atpB*–*rbcL* intergenic region in the plastid genome and the two internal transcribed spacers (ITSs) of the ribosomal RNA gene in the nuclear genome. The regions

are non-coding and variations therein are thought to be neutral to evolution.⁸ Hence, the regions are routinely analyzed in the studies of plant diversity and phylogeny.⁸

We have obtained the following findings so far. *Ligularia tongolensis* (Franch.) Hand.-Mazz., *Ligularia cymbulifera* (W.W. Smith) Hand.-Mazz., and *Ligularia atroviolacea* (Franch.) Hand.-Mazz., all belonging to the section *Corymbosae*, were close to one another with respect to the composition of furanoeremophilanes and the nucleotide sequence of the *atpB*–*rbcL* region.⁹ However, while *L. tongolensis* was found to be diverse in both indices, *L. cymbulifera* was uniform in both. More recently, we have found that samples of *Ligularia pleurocaulis* (Franch.) Hand.-Mazz. of northwestern Yunnan and those in southwestern Sichuan were different.¹⁰ 3β-Angeloyloxyfuranoeremophil-1(10)-en-6β-ol and furanoligularenone were the characteristic components, respectively, for the Yunnan and the Sichuan specimens. The *atpB*–*rbcL* sequence variation was not correlated with the chemical composition, but the ITS variation was.

Our present focus is on *Ligularia virgaurea* (Maxim.) Mattf. Both *L. virgaurea* and *L. pleurocaulis*, one of our previous subjects, belong to section *Senecillis*¹¹ and inhabit alpine meadows of more than 4000 m in altitude. While *L. pleurocaulis* is extant only in the Yunnan and the Sichuan Provinces, *L. virgaurea* is widely distributed in Yunnan, Sichuan, Qinghai, and Gansu in China, and in Nepal and

Keywords: *Ligularia virgaurea*; Sesquiterpene; Furanoeremophilane; *atpB*–*rbcL*; ITS; Diversity.

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¶ Correspond with R.H. for genetic study.

Bhutan.¹¹ The morphological variation within the species is so large that three varieties, *virgaurea*, *pilosa*, and *oligocephala*, have been proposed.^{11–13} Chemical constituents in the root of *L. virgaurea* collected in Qinghai and Gansu have been studied. Shi and co-workers obtained some eremophilanoides from var. *oligocephala* from Qinghai.¹⁴ Jia and co-workers studied the chemical constituents of this species, presumably var. *virgaurea*, from Gansu,¹⁵ and isolated several benzofuranes including cacalol, a rearranged carbon skeleton of eremophilane, and a methylated derivative of it. Here, we report that *L. virgaurea* var. *virgaurea* in southwestern Sichuan Province produces furanoeremophilanes and its derivatives, some of which are new. We also report that the plant can be grouped into two on the basis of the chemical composition and the ITS sequences.

2. Results

Eleven samples of *L. virgaurea* var. *virgaurea* were collected in southwestern Sichuan Province (Table 1 and Fig. 1). The roots of each sample were extracted with ethanol and the alcoholic solutions were subjected to Ehrlich's test on TLC plates. Two patterns of the Ehrlich-positive spots were observed. Four samples (samples 2, 5, 7, and 11; designated hereafter as type 1) showed two major components at $R_f=0.45$ and 0.53 (hexane–AcOEt=7/3) with yellow Ehrlich coloring, which implied the presence of furanoeremophilanes with some functional group. The other seven samples (samples 1, 3, 4, 6, 8, 9, and 10; type 2) showed several pink spots on TLC. The major spots appeared at $R_f=0.54$ and 0.59. The pattern was almost the same for all the seven samples, indicating that their composition of furanoeremophilanes was similar. The distribution of the two types did not coincide with the geographical distribution of the samples (Table 1 and Fig. 1).

Furanoeremophilanes and the related compounds were isolated and their structures were determined. The two components observed for the type-1 samples were purified and identified as new compounds, **1** and **2**. These two compounds were the major constituents in the type-1 samples (**1**: $R_f=0.53$, **2**: $R_f=0.45$). Related new enones **3** and **4** as well as a new lactone **5** and a known lactone **6**¹⁴ were also

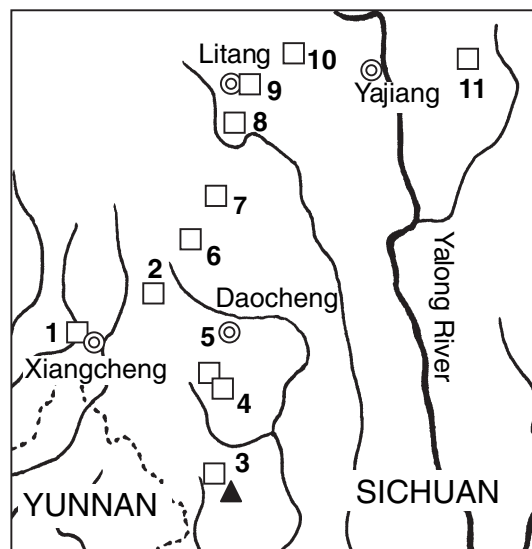


Figure 1. Locations where samples of *L. virgaurea* var. *virgaurea* (open squares) were collected. Filled triangles and double circles indicate major peaks and major cities, respectively.

isolated from the type-1 samples as minor constituents. The Ehrlich-positive components in the type-2 samples included four furanoeremophilanes: ligularol (=petasalbin, **7**),^{3a,16} its methyl ether **8**,^{5b} ethyl ether **9**,^{7,17} and furanoeremophilane-6 β ,10 β -diol (**10**).^{3b} Compounds **7** ($R_f=0.54$) and **8/9** ($R_f=0.59$) were the major Ehrlich-positive components. Compound **9** is considered to be an artifact generated during the ethanol extraction process. Eight non-furano types of compounds **11–18** were also isolated from these samples, among which **11** and **14** were new. Compounds **12**¹⁸ and **13**¹⁹ had been isolated from *Petasites japonicus* subsp. *giganteus*. Although compound **15** has not been reported, this compound seems to be an artifact during ethanol extraction.¹¹ Compounds **16**,^{3g} **17**,²⁰ and **18**²¹ had been known. The structures of the seven new compounds, **1–5**, **11**, and **14**, were determined as follows (Charts 1 and 2).

The molecular formula of compound **1**, named as virgaurenone A, was determined as $C_{20}H_{24}O_4$ by HRCIMS. The IR spectrum indicated the presence of two carbonyl moieties (1720, 1680 cm^{-1}). 1H and ^{13}C NMR data are shown in Tables 2 and 3, respectively. The ^{13}C NMR spectrum gave 20 peaks including 10 sp^2 carbons, two of which were due to carbonyl groups (δ 196.8, 167.1). The resonance at δ 6.84 was assigned to the α proton of a tri-substituted furan ring, which was supported by four signals at δ 118.3 (C), 120.1 (C), 139.6 (CH), and 148.6 (C). The substitution of an angelate group was suggested by the signals of methyl groups at δ 1.98 (dq) and 1.82 (quint), and carbon signals at δ 167.1 (CO), 128.5 (C), 140.2 (CH), 20.7 (Me), and 15.9 (Me). The HMBC spectrum showed correlations from the methyl group at C-4 into C-3, C-4, and C-5, from the methyl group at C-5 into C-4, C-5, C-6, and C-10, and from the methyl group at C-11 into C-7, C-11, and C-12. With the results of a COSY experiment, this compound was established to possess the eremophilane skeleton and

Table 1. Collection locality and *atpB-rbcL* genetic type of *L. virgaurea* samples

Sample	Locality	Elevation (m)	Ehrlich type ^a	<i>atpB-rbcL</i>	ITS clade ^b
1	Xiangcheng	4100, 4300 ^c	2	G-A11	A
2	Wumingshan	4000	1	G-A10	B
3	Yading	4000	2	G-A9	A
4	Daocheng	4100	2	G-A11	A
5	Daocheng	4400	1	G-A10	B
6	Haizishan	4300	2	G-A11	A
7	Haizishan	4400	1	G-A10	B
8	Jiawa	3800	2	G-A11	A
9	Litang	4100	2	G-A12	A
10	Honglong	4300	2	G-A11	A
11	Gaoersishan	4200	1	A-A10, 344G	B

^a Type 1=yellow color; type 2=pink color. See text.

^b See Table 4.

^c Collected at two locations close to each other. The chemical components were judged to be the same based on TLC. The DNA sequences were the same.

¹¹ Although compound **15** seems to be an artifact, the data were presented in Section 5, because a search for this compound with SciFinder made no hit and we considered the data to be worth inclusion for future comparison.

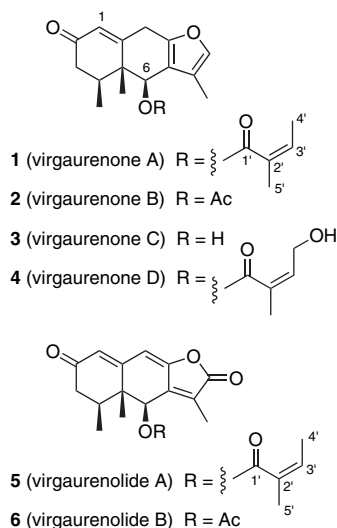


Chart 1. Chemical constituents of type-1 samples of *L. virgaurea* var. *virgaurea*.

the angelate moiety at C-6. The stereochemistry was established as shown in the formula, since NOE was detected between H-6 and H-4, and between H-9 β and H-14.

The ^1H NMR spectrum of compound **2**, virgaurenone B, $\text{C}_{17}\text{H}_{20}\text{O}_4$, was very similar to that of compound **1** except for the presence of an acetyl group and the absence of an angeloyl group (Table 2). The IR spectrum indicated the presence of carbonyl groups (1730 , 1680 cm^{-1}). Because a singlet proton at δ 6.18 had a correlation into C-1', an acetoxy group (δ 1.70) was determined to be substituted at C-6. The enone moiety (1(10)-en-2-one) was present as in **1**, which was confirmed by the 2D experiment. The stereochemistry was determined by the NOESY spectrum. Therefore, the structure was established as depicted in the formula. The sample nicely crystallized from EtOAc and the structure was solved by X-ray crystallography to support the structure deduced as above (Fig. 2) (see Section 5).

Compound **3** (virgaurenone C) showed a quasi-molecular ion peak at m/z 247 (CIMS) and an absorption of a hydroxy group at 3440 cm^{-1} in its IR spectrum. The ^{13}C NMR spectrum displayed 15 peaks including a carbonyl group (δ 197.0, Table 3). These data implied that compound **3** should contain the basic terpene part of both virgaurenones A and B.

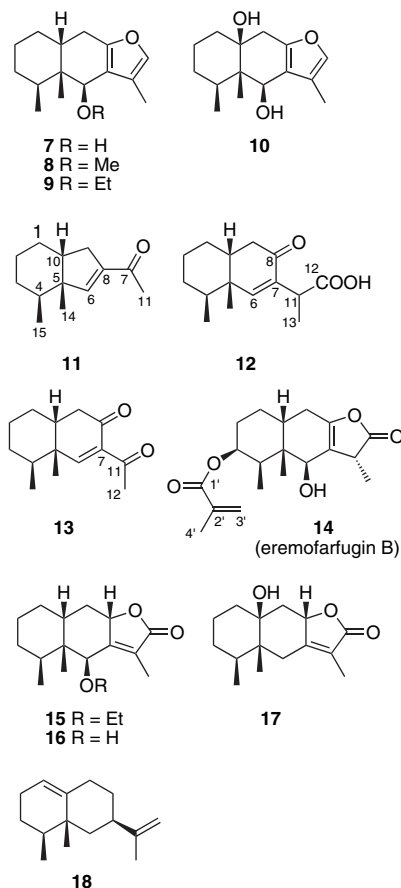


Chart 2. Chemical constituents of type-2 samples of *L. virgaurea* var. *virgaurea*.

Actually, 2D NMR, especially HMBC, supported this assumption. The NOESY spectrum confirmed the stereochemistry as depicted in the formula, since correlations between H-6 and H-4, and between H-9 β and H-14 were observed.

All CD spectra of **1–3**, indicated positive absorptions around 240 nm, which were due to π – π^* transitions in α,β -unsaturated enone systems.²² Thus, the absolute configurations of these compounds should be as depicted in the formula, since the conformation of each molecule was indicated by the NOESY spectra and the helicity attributed to this system must be clockwise.

Table 2. ^1H NMR data of compounds **1–5** (600 MHz, C_6D_6)

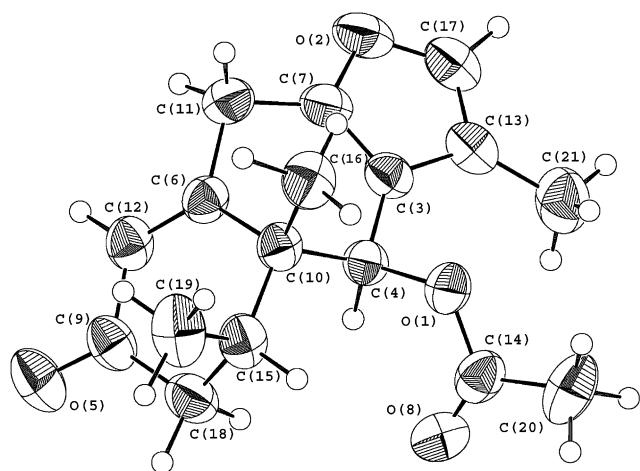
No.	1		2		3		4		5	
1	5.69	d, 1.8	5.68	d, 1.2	5.69	d, 1.6	5.67	d, 1.9	5.735	s
3 α	2.71	dd, 16.2, 4.5	2.69	dd, 16.2, 5.4	2.25	dd, 15.9, 4.4	2.66	dd, 16.0, 4.7	2.02	ddd, 17.4, 4.4, 1.1
3 β	2.10	dd, 16.2, 4.5	2.12	dd, 16.2, 4.2	2.02	dd, 15.9, 6.6	2.09	dd, 16.0, 4.1	1.93	dd, 17.4, 13.4
4	1.94	m	1.91	m	1.94	m	1.87	m	1.78	dqd, 13.4, 6.6, 4.4
6	6.34	dq, 2.4, 1.2	6.18	dq, 2.4, 1.2	4.31	br d, 6.6	6.25	dq, 2.5, 1.4	5.86	q, 1.9
9 α	2.73	d, 17.4	2.71	d, 17.6	2.71	d, 17.6	2.70	d, 17.3	5.30	s
9 β	3.12	br d, 17.4	3.10	br d, 17.6	3.08	d, 17.6	3.08	br d, 17.3	—	
12	6.84	s	6.85	s	6.87	s	6.81	s	—	
13	1.78	d, 1.2	1.72	d, 1.2	1.93	d, 1.4	1.71	d, 1.4	1.56	d, 1.9
14	0.90	s	0.87	s	0.65	s	0.85	s	0.74	s
15	0.70	d, 6.6	0.72	d, 7.2	0.83	d, 7.1	0.68	d, 6.8	0.57	d, 6.6
2'	—		1.70	s	—		—		—	
3'	5.78	qq, 7.8, 1.8	—		—		6.05	tq, 5.2, 1.6	5.740	qq, 7.1, 1.4
4'	1.98	dq, 6.6, 1.2	—		—		4.49	m	1.89	dq, 7.1, 1.4
5'	1.82	quint, 1.2	—		—		1.74	q, 1.6	1.68	quint, 1.4

Table 3. ^{13}C NMR data of compounds **1–5** (150 MHz, C_6D_6)

No.	1	2	3	4	5
1	126.9	126.9	126.5	126.9	129.1
2	196.8	196.9	197.0	196.7	195.4
3	42.7	42.7	42.8	42.6	43.5
4	33.9	33.9	34.9	33.9	38.0
5	44.4	44.3	45.0	44.3	44.3
6	69.7	70.2	71.1	70.4	73.1
7	118.3	118.0	120.6	117.9	143.7
8	148.6	148.6	147.7	148.7	151.2
9	31.4	31.4	31.2	31.3	107.0
10	156.0	156.1	158.9	155.8	157.4
11	120.1	120.0	120.3	119.9	125.5
12	139.6	139.6	139.3	139.7	169.1
13	8.7	8.6	9.2	8.6	8.4
14	17.2	16.9	14.9	17.1	13.8
15	16.0	15.9	16.5	15.9	16.9
1'	167.1	170.2	—	167.0	166.0
2'	128.2	20.4	—	126.5	126.4
3'	140.2	—	—	147.4	142.8
4'	15.9	—	—	61.0	16.0
5'	20.7	—	—	19.8	20.3

The molecular formula of compound **4** (virgaurenone D), determined as $\text{C}_{20}\text{H}_{24}\text{O}_5$, indicated that a C_5 ester should be attached to the eremophilane skeleton, since the ^1H NMR spectrum of **4** was very similar to those of **1**, **2**, and **3** (see Table 2). From careful analysis of the HMBC spectrum, it was inferred that the terpene part was the same as that of **3**, but the ester part was not an angelate. The methyl group at δ 1.74 (H-5') had correlations into C-1', C-2', and C-3'. The proton at δ 6.05 (H-3') was coupled with the protons at δ 4.49 (2H, H-4'). Therefore, this ester should be 4-hydroxy-2-methyl-2-butenate and the whole structure was established as depicted in the formula, including the stereochemistry.

The molecular formula of virgaurenone A (**5**) was $\text{C}_{20}\text{H}_{22}\text{O}_5$ and the degree of unsaturation was 10. The ^{13}C NMR spectrum indicated 11 sp^2 carbons, three of which were due to carbonyl groups. The IR spectrum showed absorptions of a lactone (1780 cm^{-1}), an ester (1720 cm^{-1}), and a carbonyl (1660 cm^{-1}) group. The HMBC spectrum clearly indicated the presence of a 1(10)-en-2-one moiety, which was further conjugated to the lactone ring. Therefore, the HMBC spectrum indicated that the basic skeleton must be 2-oxoeremophil-1(10),7(11),8-triene-12,8-olide. This

**Figure 2.** The X-ray crystal structure of **2**.

was presumably produced from the corresponding furanoeremophilane (**1**) by oxidation in the plant. The stereochemistry was determined as depicted in the formula, since NOE was observed between H-6 and H-4. The acetylated compound **6** (virgaurenone B) had been isolated recently from *L. virgaurea* var. *oligocephala*.¹⁴

Compound **11** showed a molecular ion peak at m/z 192, whose molecular formula was calculated as $\text{C}_{13}\text{H}_{20}\text{O}$ by HRMS. The ^{13}C NMR spectrum indicated the presence of a carbonyl group (δ 195.6), which was supported by the absorption at 1670 cm^{-1} in the IR spectrum. An acetyl group was detected at δ 1.97 (3H, s) in the ^1H NMR spectrum. The ^1H NMR spectrum also showed the presence of a *tert*-methyl and a *sec*-methyl group as well as one proton absorption at δ 6.30 assignable to an olefinic proton. Since the total number of ^{13}C NMR signals was 13 and the degree of unsaturation was 4, this compound was judged to be a bicyclic dinor-sesquiterpene. The HMBC spectrum indicated correlations from the methyl group at C-5 into C-4, C-5, C-6, and C-10. The methyl group at C-4 had correlations into C-3, C-4, and C-5. These results indicated a dinor-eremophilane skeleton. The sequence of protons of H-4, H-3, H-2, H-1, and H-10 were indicated by the COSY spectrum. NOE was detected between H-10 and H-14 and indicated that two carbocycles were *cis*-fused. Thus, the 12,13-dinor-eremophilane skeleton was established. Similar compounds with an oxygen functionality at C-3 had been known.^{5e}

Compound **14** indicated a characteristic absorption at 1800 cm^{-1} in its IR spectrum, suggesting the presence of an enol γ -lactone as preceded in eremofarugin A.^{5c,5d} The molecular formula was determined to be $\text{C}_{19}\text{H}_{26}\text{O}_5$ by HRMS. Both the ^1H and the ^{13}C NMR spectra obtained at room temperature showed very broad peaks and were not analyzable. Therefore, spectra were obtained at 50°C in C_6D_6 and analyzed. The HMBC spectrum indicated correlations from the methyl group at C-5 to C-4, C-5, C-6, and C-10, from the methyl group at C-4 to C-3, C-4, and C-5, and from the methyl group at C-11 to C-7, C-11, and C-12. A hydroxy group (3500 cm^{-1}) must be at C-6 (δ 65.3), while an acyl group should be at C-3 (δ 72.3), because H-3 (δ 5.19) had a correlation into C-1' (δ 166.5). The acyl group was an α -methylacrylate, because a methyl group at C-2' had correlations into C-1', C-2', and C-3' in the HMBC spectrum. The stereochemistry was determined as depicted in the formula by the NOESY spectrum. One of the authors previously isolated eremofarugin A from *Farfugium japonicum*, which had an angelate group substituted at C-3 instead of α -methylacrylate.^{3f} Compound **14** lacks one methyl group of eremofarugin A and was named as eremofarugin B.

Purification of DNA from the samples, the amplification of the *atpB-rbcL* region, and the determination of the base sequence of the region, consisting of about 740 base-pairs, were carried out as described previously.^{9,10} Five variants, G-A9, G-A10, G-A11, G-A12, and A-A10, 344G, according to our designation,^{††} were identified (Table 1). The base

^{††} The 28th base was A or G and the number of an A stretch around the 510th base was 9, 10, 11, or 12. The variant type was designated by the combination of A/G and A9/A10/A11/A12, such as G-A12; 344G denotes the 344th position to be G in place of T in the other samples. See Ref. 9.

ITS1
 TCGAAACCTGCATAGCAGAACGACCCGTGAACATGTAAACAATCGGGTGCTCTGGTA 60
 TCGGGCTCTTGTTCGATTAATTGGATGCCTTGTGATGTGCGTCTTTGGTCAGCCCTTG 120
 GGTCTTAAGGACGTACATTTGGCGCAACAACACCCCGGCACGGCATGTGCCAAGGAA 180
 AATTAAACTTAAGAAGGGCTGTACCATGCTTCCCGTTTGGCGGGTTGTGATGGGACGT 240
 GGCTCTTTTATAATCA 256

ITS2
 ATCGCGTCGCCCCACCAACGCTCTCGATGAGRATGCTTGGATGTGGCGGAGATTGGT 60
 CTCCCGTTCTTAYGGTGGCTTGGCTAAACAGGAGTCCCTTCGACGGACGACGATTA 120
 GTGGTGGTTGACAAGACCTCTTATCAAGTTGTGCGTCTAAGGAGCAAGGAATGTCTCT 180
 TCAATGACCCCAATGTGTCTGCTGTGACGATGCTTCGACC 221

Figure 3. The nucleotide sequences of ITS1 and ITS2 of sample 5. They have been deposited to DDBJ/EMBL/GenBank together with the sequence of the 5.8S rRNA region between the ITSs and sequences flanking the ITSs (accession number AB245093).

sequences of the ITSs of the ribosomal RNA gene were also determined as previously (Fig. 3).¹⁰ Multiple bases were observed at some positions, as thousands of copies of the gene are present in a cell and there can be variations among the copies.⁸ Sequence variation among the samples was observed at a number of base positions, as summarized in Table 4. Analysis of the variation using the PHYLIP program package²³ indicated that the samples can be grouped into two clades, tentatively designated A and B in Tables 1 and 4, with a bootstrap value of 95.5% at the branch of the clades.

3. Discussion

In the present study, *L. virgaurea* was examined with respect to the chemical composition and the nucleotide sequences of the *atpB-rbcL* region and the ITSs of the ribosomal RNA gene. Seven new compounds, virgaurenones A–D (1–4), virgaurenolide A (5), compound 11, and eremofarugin B (14), were obtained together with several known compounds. All the isolated compounds had the eremophilane or a related skeleton: Compounds 1–4 and 7–10 were furanoeremophilanes, and 5, 6, 12, 14–17 were their oxidized compounds; compound 13 had only 14 carbons and was considered to be a decarboxylated product of 12; compound 11 was presumably a decarbonylatively ring-contracted product of 13. Since eremophilanolides and related benzofurans have been obtained from *L. virgaurea* samples collected in Qinghai¹⁴ and in Gansu,¹⁵ production of eremophilanes

must be common in this species. The present results showed that furanoeremophilanes are also produced by this species.

The presence of intra-specific variation was apparent. Four of the 11 collected samples (type 1) contained virgaurenones A–D (1–4) and their oxidized compounds (5 and 6). In contrast, these compounds were absent in the other seven samples (type 2). Petasalbin methyl and ethyl ethers (8 and 9) and 8-oxoeremophil-6-en-12-oic acid (12) were common to these samples. Types 1 and 2 can be considered quite different from each other, since no common components were isolated from both the types. This contrasts with our previous observation with *L. pleurocaulis*, in which two types had several compounds in common.¹⁰ Interestingly, all compounds isolated from the type-1 samples had a ketone group at C-2 position. Virgaurenones A–D are the first examples of furanoeremophilanes having the 1(10)-en-2-one system, in which the hydrogen at 9-position is highly acidic.

Intra-specific variation was also observed in nucleotide sequences, both in the *atpB-rbcL* region and in the ITSs. Although no clear correlation was observed between the chemical composition and the *atpB-rbcL* sequence, grouping based on the composition (types 1 and 2) and that based on the ITS sequences (clades A and B) agreed (Table 1). We have reported a similar finding for *L. pleurocaulis*, as mentioned in Section 1.¹⁰ The present agreement further supports our previous interpretation¹⁰ that the chemical–genetic correlation is stronger for the ITSs of the rRNA gene because the terpene-related genes and the rRNA gene are encoded in the nuclear genome. Thus the chemical diversity in *L. virgaurea* seems to result from a difference(s) of a genetic character, but not from an environmental difference(s).

Interestingly, classification of the *L. virgaurea* samples on the basis of the composition type and the ITS clade showed no geographic division, while the chemical–genetic grouping of *L. pleurocaulis* agreed with geographic division.¹⁰ Within the Daocheng/Litang area where we collected samples of both species, *L. pleurocaulis* was found to be almost uniform,¹⁰ whereas *L. virgaurea* was diverse. It is also noteworthy that *atpB-rbcL* sequence of sample 11 is rather different from the others. This fact and the difference of the

Table 4. Variations in ITS1 and ITS2 in *L. virgaurea* var. *virgaurea*

Sample	Base position																					Clade		
	ITS1											ITS2												
	11	65	66	74	124	130	132	134	135	235	240	32	34	73	84	91	162	168	175	179	188		217	220
1	C	A	C	C	C	R	Y	Y	Y	S	C	G	A	T	R	C	A	A	A	A	C	C	C	A
3	Y	R	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	M	A	A	R	A	Y	C	C	A
4	Y	A	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
6	Y	A	C	Y	C	R	Y	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
8	C	A	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
9	C	A	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
10	C	A	C	Y	C	R	C	T	Y	G	C	G	A	T	R	C	A	A	A	A	C	C	C	A
2	C	G	Y	C	C	G	C	T	C	G	T	A	R	Y	G	C	A	W	G	C	C	Y	Y	B
5	C	G	C	C	C	G	C	T	C	G	T	A	R	Y	G	C	A	A	G	C	C	C	C	B
7	C	G	C	C	Y	G	C	T	C	G	T	A	R	Y	G	C	A	A	G	C	C	Y	C	B
11	C	G	C	C	C	G	C	T	C	G	T	A	A	C	G	C	W	W	G	C	C	C	C	B

The base numbering is for each ITS region as in Figure 2. All the multiplicities are listed irrespective of the relative occurrences of the two bases. Y=C+T; R=A+G; M=A+C; S=C+G; W=A+T.

chemical composition between a previous¹⁵ and the present studies suggest that *L. virgaurea* var. *virgaurea* may harbor further diversity outside the areas of the current survey.

4. Conclusion

Seven new compounds were isolated from *L. virgaurea* var. *virgaurea* collected in southwestern Sichuan Province of China, and their structures were established. Four of them, virgaurenones A–D were the first examples of the furano-eremophilane having a 1(10)-en-2-one moiety. The presence of diversity in the species was demonstrated. The samples could be classified into two groups on the bases of their composition of the extracted compounds. The base variations in the ITSs also showed that the samples can be grouped into two, and this grouping was in concord with the chemical one. These results suggest that the chemical difference has a genetic origin.

5. Experimental

5.1. General

Specific rotations and CD spectra were measured on a JASCO DIP-1000 and a JASCO J-725 auto recording polarimeter; IR spectra, on a JASCO FT/IR-5300 spectrophotometer; ¹H and ¹³C NMR spectra, on a Varian Unity 600 (600 MHz and 150 MHz, respectively) and a JEOL ECP 400 (400 MHz and 100 MHz, respectively) spectrometer. Mass spectra, including high-resolution ones, were recorded on a JEOL JMS-700 MStation. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer using a DIP image plate. Chemcopak Nucleosil 50-5 (4.6×250 mm) with a solvent system of hexane–ethyl acetate was used for HPLC (a JASCO pump system). Silica gel 60 (70–230 mesh, Fuji Sylisia) was used for column chromatography. Silica gel 60 F₂₅₄ plates (Merck) were used for TLC. Determination of the DNA sequences of the *atpB*–*rbcL* intergenic region and the ITSs was carried out as described previously.^{9,10}

5.2. Plant materials

Samples of *L. virgaurea* var. *virgaurea* were collected in August 2003 and 2005 at 11 locations (Table 1 and Fig. 1). Samples 2, 3, 4, and 6 were collected in 2003, and samples 1, 5, and 7–11 were collected in 2005. Each plant was identified by Xun Gong, one of the authors.

5.3. Ehrlich's test

The root of each plant (about 10 g) was harvested and extraction with ethanol was started immediately without drying. Extraction was continued at room temperature for several days. After filtering and without concentrating, extracts were subjected to TLC (Kieselgel 60 F₂₅₄, layer thickness 0.2 mm) using hexane–ethyl acetate (7:3) as the solvent. The TLC plate was dipped in a 1% solution of *p*-dimethylaminobenzaldehyde in ethanol. The plate was dried and then dipped in a 1 M solution of hydrochloric acid in aqueous ethanol and the resultant coloring was photographed.

5.4. Extraction, purification, and structure determination

For the samples collected in 2003, the collected roots of *L. virgaurea* were cut into small pieces without drying, and immediately extracted with EtOH at room temperature. The extract was filtered and concentrated to afford an oily residue with an aqueous phase. AcOEt was added to this oil/aqueous mixture and the organic layer was recovered. Evaporation of the solvent afforded an oily residue, to which water soluble starch was added for handling purpose. For the samples collected in 2005, the roots were dried and extracted with AcOEt at room temperature. Oily extracts were obtained by the standard method.

Components of the extract of sample 2 (1.98 g with starch, extracted from 65 g of fresh root) were separated by silica gel (50 g) column chromatography (hexane–EtOAc, in gradient) to give **1** (46.1 mg) and **2** (37.5 mg). Compound **2** was recrystallized from EtOAc to give rectangular crystals.

Components of the extract of sample 6 (5.4 g with starch, extracted from 95 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to give five fractions. The second fraction (29 mg) was further subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 1 mL/min, hexane–EtOAc (95:5)) to afford **9** (3.6 mg), **8** (4.7 mg), and **11** (6.6 mg). The fourth fraction (69.7 mg) was subjected to silica gel column chromatography (hexane–EtOAc, in gradient) followed by HPLC (Nucleosil 50-5, 4.6×250 mm, 3 mL/min, hexane–EtOAc (97:3)) to give **13** (2.1 mg)¹⁹ and **15** (1.3 mg). The fifth fraction (831 mg) was further subjected to silica gel column chromatography (CHCl₃–EtOAc, in gradient) to give **12** (140.8 mg).¹⁸

Components of the extract of sample 3 (4.3 g with starch, extracted from 120 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford 10 fractions. The first fraction (102.1 mg), further separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (95:5)), contained petasabin methyl ether (**8**) (45.5 mg) and ethyl ether (**9**) (41.4 mg). The seventh fraction (59 mg) was subjected to silica gel column chromatography (hexane–EtOAc, in gradient) followed by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to give **10** (4.6 mg),^{3b} **14** (1.1 mg), and **13** (1.0 mg).¹⁹ The ninth fraction (54.6 mg) was subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **14** (4.6 mg). The 10th fraction (326.4 mg) was subjected to silica gel column chromatography (CHCl₃–EtOAc, in gradient) to give **12** (39.5 mg).

The extract of sample 4 (1 g with starch, extracted from 45 g of fresh root) was subjected to silica gel column chromatography (hexane–EtOAc, in gradient) and the resulting 10 fractions were further subjected to a combination of column chromatography and HPLC. However, the compounds were very labile and no compound was isolated in pure. Eremophila-1(10),11-diene (**18**) alone was detected by GC–MS.

Components of the extract of sample 5 (818 mg, extracted from 11 g of fresh root) were separated by silica gel column

chromatography (hexane–EtOAc, in gradient) to afford six fractions. The second fraction was virgaurenone A (**1**) (190.9 mg) and the third fraction was virgaurenone B (**2**) (195.6 mg). The fourth fraction (19.3 mg) was subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to give virgaurenone A (**5**) (4.3 mg). The fifth fraction (17.8 mg) was subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **6** (5.0 mg).¹⁴

Components of the extract of sample 7 (464 mg, extracted from 5 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford seven fractions. The second fraction was virgaurenone A (**1**) (50.4 mg) and the fourth fraction was virgaurenone B (**2**) (79.6 mg). The third fraction (11.3 mg) was further separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (9:1)) to afford **1** (1.0 mg) and **2** (2.3 mg).

Components of the extract of sample 8 (699 mg, extracted from 5 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford seven fractions. The third fraction was ligularol (**7**) (17.0 mg). The sixth fraction (62.2 mg) was purified by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **16** (1.3 mg). The seventh fraction (263 mg) was further separated by silica gel column chromatography (CHCl₃–EtOAc, in gradient) to give **17** (24.1 mg).²⁰

Components of the extract of sample 10 (1.3 g, extracted from 13 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford seven fractions. The second (39.3 mg) and third (21.8 mg) fractions were purified by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (95:5)) to afford ligularol (**7**) (8.2 mg). The fifth fraction (95.7 mg) was further separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **16** (2.5 mg) and **17** (19.7 mg). The seventh fraction (158 mg) was separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to give **12** (6.7 mg).

Components of the extract of sample 11 (1.3 g, extracted from 15 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford nine fractions. The fourth fraction was virgaurenone A (**1**) (160.4 mg) and the fifth fraction was virgaurenone B (**2**) (155.4 mg). The eighth fraction (24.4 mg) was purified by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (70:30)) to afford virgaurenone C (**3**) (5.8 mg), **6** (0.4 mg), and virgaurenone D (**4**) (8.9 mg).

5.4.1. 6 β -Angeloyloxyfuranoeremophil-1(10)-en-2-one (1). A pale yellow oil; [α]_D²⁰ +19.0 (*c* 1.15, EtOH); MS (CI) *m/z* 329 [M+H]⁺, 271, 269, 229 (base), 228, 186, 83; HRMS (CI) obsd 329.1749 [M+H]⁺ Calcd for C₂₀H₂₅O₄ 329.1753; FTIR (KBr) 1720, 1680, 1620 cm⁻¹; CD [θ]_{243 nm} +18,600; see Tables 2 and 3 for ¹H and ¹³C NMR, respectively.

5.4.2. 6 β -Acetoxylfuranoeremophil-1(10)-en-2-one (2). Pale yellow rectangular crystals; mp 142.5–143.5 (from

EtOAc); Analysis obsd C: 70.62, H: 7.08%, Calcd for C₁₇H₂₀O₄ C: 70.81, H: 6.99%; [α]_D¹⁹ –7.79 (*c* 0.94, EtOH); MS (CI) *m/z* 289 [M+H]⁺, 246, 229 (base), 213, 186, 175, 123; HRMS (CI) obsd 289.1434 [M+H]⁺ Calcd for C₁₇H₂₁O₄ 289.1440; FTIR (KBr) 1740, 1680, 1620 cm⁻¹; CD (EtOH) [θ]_{214 nm} –17,900, [θ]_{238 nm} +25,700; see Tables 2 and 3 for ¹H and ¹³C NMR, respectively. X-ray data: orthorhombic, *P*2₁2₁2₁, *a*=8.9140 (3) Å, *b*=12.4900 (5) Å, *c*=13.9270 (4) Å, α =90.00°, β =90.00°, γ =90.00°, *V*=1550.57 (9) Å³, *Z*=4; Mo K α radiation, λ =0.71073; 2849 measured reflections, 2840 independent reflections, 2764 observed reflections, θ_{\max} =25.73°; refinement on *F*², full matrix least-squares refinement, *R*(all)=0.0459, *wR*(ref)=0.1162, *S*(ref)=1.161, 2840 reflections; extinction correction, *SHELXL*, extinction coefficient=0.113 (8). Crystallographic data for compound **2** have been deposited at the Cambridge Crystallographic Data Center as supplementary publication number CCDC 295246. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/data_request/cif, or by mailing to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: data_request@ccdc.cam.ac.uk].

5.4.3. 6 β -Hydroxyfuranoeremophil-1(10)-en-2-one (3). A pale yellow oil; [α]_D²¹ –24.3 (*c* 0.58, EtOH); MS (CI) *m/z* 247 [M+H]⁺, 229 (base); HRMS (CI) obsd 247.1328 [M+H]⁺, Calcd for C₁₅H₁₉O₃ 247.1334; FTIR (KBr) 3440, 1670, 1620 cm⁻¹; CD (EtOH) [θ]_{222 nm} –10,000, [θ]_{248 nm} +11,500, [θ]_{320 nm}+3600; see Tables 2 and 3 for ¹H and ¹³C NMR, respectively.

5.4.4. 6 β -(*Z*-4'-Hydroxy-2-methyl-2-butenyl)oxyfuranoeremophil-1(10)-en-2-one (4). A pale yellow oil; [α]_D²⁰ –7.1 (*c* 0.89, EtOH); MS (FAB) *m/z* 345 [M+H]⁺, 154 (base), 136; HRMS (FAB) obsd 345.1722 [M+H]⁺, Calcd for C₂₀H₂₅O₅ 345.1702; FTIR (KBr) 3400, 1710, 1670, 1630 cm⁻¹; see Tables 2 and 3 for ¹H and ¹³C NMR, respectively.

5.4.5. 6 β -Angeloyloxy-2-oxoeremophil-1(10),7(11),8-triene-12,8-olide (5). A pale yellow oil; [α]_D²⁰ –344 (*c* 0.43, EtOH); MS (CI) *m/z* 343 [M+H]⁺ (base), 245; HRMS (CI) obsd 343.1547 [M+H]⁺, Calcd for C₂₀H₂₃O₅ 343.1545; FTIR 1780, 1720, 1660, 1640 cm⁻¹; CD (EtOH) [θ]_{223 nm} –1460, [θ]_{266 nm}+3400, [θ]_{308 nm} –5900; see Tables 2 and 3 for ¹H and ¹³C NMR, respectively.

5.4.6. 1-[(3*aR*,4*S*,7*aR*)-3*a*,4,5,6,7*a*-Hexahydro-3*a*,4-dimethyl-1*H*-inden-2-yl]ethanone (11). A colorless oil; [α]_D²⁰ +5.6 (*c* 0.49, EtOH); MS *m/z* 192 [M]⁺ 177, 149 (base), 136, 122, 107, 93, 79; HRMS obsd 192.1523 [M]⁺, Calcd for C₁₃H₂₀O 192.1515; FTIR (KBr) 1670 cm⁻¹; ¹³C NMR (150 MHz, C₆D₆) δ 17.3 (C-14), 17.4 (C-15), 22.3 (C-2), 24.5 (C-1), 26.0 (C-11), 29.4 (C-3), 33.7 (C-9), 37.0 (C-4), 46.6 (C-10), 50.0 (C-5), 144.0 (C-8), 153.2 (C-6), 195.6 (C-7); ¹H NMR (600 MHz, C₆D₆) δ 0.67 (3H, d, *J*=6.6 Hz, H-15), 0.84 (3H, s, H-14), 0.86 (1H, m, H-3), 1.09 (1H, m, H-4), 1.11 (1H, m, H-3), 1.18 (1H, m, H-2), 1.31 (1H, m, H-2), 1.34 (1H, m, H-1), 1.42 (1H, m, H-1), 1.75 (1H, m, H-10), 1.97 (3H, s, H-11), 2.43 (1H, ddd, *J*=16.0, 11.3, 2.4 Hz, H-9 α), 2.57 (1H, dd, *J*=16.0, 8.1 Hz, H-9 β), 6.30 (1H, d, *J*=2.4 Hz, H-6).

5.4.7. 6 β -Hydroxy-3 β -(2'-methylacryloyloxy)-11 β H-eremophil-7-en-12,8-olide (14). A colorless oil; $[\alpha]_D^{18}$ –64.2 (c 0.11, EtOH); MS (CI) m/z 335 $[M+H]^+$, 317, 249 (base), 231, 195, 109; HRMS (CI) obsd 335.1860 $[M+H]^+$, Calcd for $C_{19}H_{27}O_5$ 335.1859; FTIR (KBr) 3400, 1800, 1710 cm^{-1} ; CD $\Delta\epsilon_{217\text{ nm}}$ –19.2; ^{13}C NMR (150 MHz, C_6D_6) δ 7.8 (C-15), 14.3 (C-13), 18.3 (C-4'), 19.0 (C-14), 25.4 (C-9), 25.9 (C-2), 29.5 (C-1), 32.8 (C-10), 35.0 (C-4), 39.1 (C-11), 42.0 (C-5), 65.3 (C-6), 72.3 (C-3), 116.1 (C-7), 125.2 (C-3'), 137.1 (C-2'), 145.1 (C-8), 166.5 (C-1'), 179.0 (C-12); 1H NMR (600 MHz, C_6D_6) δ 0.67 (3H, br s, H-14), 0.79 (3H, d, $J=7.1$ Hz, H-15), 1.10 (3H, d, $J=7.7$ Hz, H-13), 1.85 (3H, dd, $J=1.3$ Hz, 0.8, H-4'), 3.04 (1H, br quint, H-11), 4.33 (1H, br s, H-6), 5.19 (1H, m, H-3), 5.22 (1H, quint, $J=1.3$ Hz, H-3'), 6.16 (1H, quint, $J=0.8$ Hz, H-3'). Other 1H NMR signals could not be assigned due to broadening, see Ref. 3f.

5.4.8. 6 β -Ethoxyeremophil-7(11)-en-12,8 α -olide (15). ^{||} A colorless oil; $[\alpha]_D^{21}$ +112.0 (c 0.10, EtOH); MS (CI) m/z 279 $[M+H]^+$ (base), 233, 169, 154, 126; HRMS (CI) obsd 279.1964 $[M+H]^+$, Calcd for $C_{17}H_{27}O_3$ 279.1960; FTIR 1760 cm^{-1} ; CD $\Delta\epsilon_{226\text{ nm}}$ +4.61 (EtOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.6, 15.3, 16.3, 20.0, 20.5, 25.9, 29.1, 30.6, 34.6, 35.1, 42.8, 64.3, 77.4, 78.0, 124.2, 159.7, 170.0; 1H NMR (600 MHz, $CDCl_3$) δ 0.77 (3H, d, $J=6.3$ Hz, H-15), 1.08 (3H, s, H-14), 1.15 (3H, t, $J=6.9$ Hz, H-2'), 1.25–1.44 (7H, m), 1.70 (1H, td, $J=14.0$, 11.5 Hz, H-9 α), 1.74 (1H, m, H-10), 1.86 (3H, d, $J=1.6$ Hz, H-13), 2.06 (1H, m, H-9 β), 2.97 (1H, dq, $J=9.2$, 7.0 Hz, H-1'), 3.39 (1H, dq, $J=9.2$, 7.0 Hz, H-1'), 4.15 (1H, br s, H-6 α), 4.94 (1H, m, H-8 β).

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References and notes

- Jeffrey, C.; Chen, Y. L. *Kew Bull.* **1984**, 39, 205–446.
- Liu, S.-W.; Deng, D.-S.; Liu, J.-Q. *Acta Phytotaxonom. Sin.* **1994**, 32, 514–524.
- For examples, (a) Ishii, H.; Tozoy, T.; Minato, H. *Tetrahedron* **1965**, 21, 2605–2610; (b) Patil, F.; Ourisson, G.; Tanahashi, Y.; Wada, M.; Takahashi, T. *Bull. Soc. Chim. Fr.* **1968**, 1047–1054; (c) Bohlmann, F.; Zdero, C. *Phytochemistry* **1980**, 19, 1550–1551; (d) Tada, M.; Moriyama, Y.; Tanahashi, Y.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1974**, 47, 1999–2002; (e) Sato, T.; Moriyama, Y.; Nagano, H.; Tanahashi, Y.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1975**, 48, 112–114; (f) Tori, M.; Shiotani, Y.; Tanaka, M.; Nakashima, K.; Sono, M. *Tetrahedron Lett.* **2000**, 41, 1797–1799; (g) Moriyama, Y.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1976**, 49, 3196–3199.
- Nagano, H.; Tanahashi, Y.; Moriyama, Y.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1973**, 46, 2840–2845.
- For examples, (a) Novotný, L.; Kotva, K.; Toman, J.; Herout, V. *Phytochemistry* **1972**, 11, 279–2799; (b) Naya, K.; Nakagawa, M.; Hayashi, M.; Tsuji, K.; Naito, M. *Tetrahedron Lett.* **1971**, 2961–2964; (c) Tori, M.; Kawahara, M.; Sono, M. *Tetrahedron Lett.* **1997**, 38, 1965–1968; (d) Tori, M.; Kawahara, M.; Sono, M. *Phytochemistry* **1998**, 47, 401–409; (e) Yaoita, Y.; Kikuchi, M. *Chem. Pharm. Bull.* **1996**, 44, 1731–1735.
- Kuroda, C.; Murae, T.; Tada, M.; Nagano, H.; Tsuyuki, T.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1982**, 55, 1228–1233.
- Kuroda, C.; Ueshino, T.; Nagano, H. *Bull. Chem. Soc. Jpn.* **2004**, 77, 1737–1740.
- (a) Savolainen, V.; Mane, J. F.; Douzery, E.; Spchiger, R. *Mol. Phylogenet. Evol.* **1994**, 3, 27–37; (b) Soltis, D. E.; Soltis, P. S. *Molecular Systematics of Plants. II DNA Sequencing*; Soltis, P. S., Soltis, D. E., Doyle, J. J., Eds.; Kluwer Academic: Boston, 1998.
- Hanai, R.; Gong, X.; Tori, M.; Kondo, S.; Ootose, K.; Okamoto, Y.; Nishihama, T.; Murota, A.; Shen, Y.; Wu, S.; Kuroda, C. *Bull. Chem. Soc. Jpn.* **2005**, 78, 1302–1308.
- Nagano, H.; Iwazaki, Y.; Gong, X.; Shen, Y.; Kuroda, C.; Hanai, R. *Bull. Chem. Soc. Jpn.* **2006**, 79, 300–304.
- Liu, S.-W.; Flora Reipublicae Popularis Sinicae; Science: Beijing, 1989; Vol. 77.
- Liu, S.-W.; Flora Qinghai; Qinghai People's: 1999; Vol. 3; pp 427–428.
- Liu, S.-W.; Ho, T.-N. *Acta Phytotaxonom. Sin.* **2000**, 38, 286–288.
- (a) Wu, Q.-X.; Shi, Y.-P.; Yang, L. *Org. Lett.* **2004**, 6, 2313–2316; (b) Wu, Q.-X.; Shi, Y.-P.; Yang, L. *Planta Med.* **2004**, 70, 479–482; (c) Wu, Q.-X.; Yang, A.-M.; Shi, Y.-P. *Tetrahedron* **2005**, 61, 10529–10535.
- (a) Wang, B.-G.; Jia, Z.-J.; Yang, X.-P. *Planta Med.* **1997**, 63, 577–578; (b) Chen, H.-M.; Wang, Y.-Y.; Mao, J.-M.; Cai, M.-S.; Jia, Z.-J. *Planta Med.* **1997**, 63, 299–302; (c) Jia, Z.-H.; Chen, H.-M. *Phytochemistry* **1991**, 30, 3132–3134.
- Yamakawa, K.; Satoh, T. *Chem. Pharm. Bull.* **1979**, 27, 1747–1752.
- Nagano, H.; Kuroda, C.; Moriyama, Y.; Tsuyuki, T.; Takahashi, T. *Bull. Chem. Soc. Jpn.* **1982**, 55, 1221–1227.
- Tori, M.; Kume, M.; Nakashima, K.; Sono, M.; Tanaka, M. *Heterocycles* **2005**, 65, 887–891.
- (a) Goto, Y.; Kojima, Y.; Nakayama, T.; Terazawa, M. *Phytochemistry* **2001**, 57, 109–113; (b) Li, Y. S.; Wang, Z. T.; Zhang, M.; Chen, J. J.; Luo, S. D. *Nat. Prod. Res.* **2004**, 18, 99–104.
- Massiot, G.; Nuzillard, J.-M.; Men-Olivier, L. L.; Acilina, P.; Benkouidera, A.; Khelifa, A. *Phytochemistry* **1990**, 29, 2207–2210.
- Křepinský, J.; Motl, O.; Dolejš, L.; Novotný, L.; Herout, V.; Bates, R. *Tetrahedron Lett.* **1968**, 9, 3315–3318.
- (a) Djerassi, C.; Riniker, R.; Riniker, B. *J. Am. Chem. Soc.* **1956**, 78, 6377–6389; (b) Gawroński, J. K. *Tetrahedron* **1982**, 38, 3–26; (c) Sneath, G. *Tetrahedron* **1965**, 21, 413–419.
- Baum, B. R. *Quart. Rev. Biol.* **1989**, 64, 539–541.