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Eremophilane, bakkane, secoeremophilane, and secobakkane sesquiterpenoids from *Ligularia virgaurea* collected in China

Yoshinori Saito [a, *], Saori Iga [b], Kie Hoshiyama [b], Katsuyuki Nakashima [b], Yasuko Okamoto [b], Chiaki Kuroda [c], Xun Gong [d], Motoo Tori [b]

[a] Graduate School of Biomedical Sciences, Nagasaki University, Bunkyo-machi, Nagasaki 852-8521, Japan
[b] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan
[c] Department of Chemistry, Rikkyo University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan
[d] Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, China

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ABSTRACT

Secobakkane B (C-6/C-7 cleaved secobakkane type aldehyde), secovirgaurenols B and C (C-8/C-9 cleaved secoeremophilane type), a 1β,10β-epoxyfuranoeremophilane, two 1β,10β-epoxyeremophilanolides, and fukinospirolide C (bakkane-type lactone), as well as 33 known compounds were isolated from three samples of *Ligularia virgaurea* collected in China. Two of the three analyzed samples were grouped in the neoadenostylone (N) type, and the rest, a mixture of the 6-hydroxyeuryopsyn (H) and cacalol (C) types, out of five chemotypes found in this species.

1. Introduction

We have been studying both the chemical constituents and DNA sequences of many *Ligularia* plants grown in China, particularly in Yunnan, Sichuan, and adjacent areas. *L. virgaurea* (Maxim.) Mattf. is an abundant species in Sichuan Province, growing in alpine meadows at around 4000 m in altitude. To date, we reported that *L. virgaurea* is an eremophilane-producing species with a large intra-specific diversity in substituents [1–6], whereas many *Ligularia* species show intra-specific diversity at various levels [6]. Five chemotypes, virgaurenone (V), ligularol (L), cacalol (C), neoadenostylone (N), and 6-hydroxyeuryopsin (H) types have been identified in this species [1,3]. However, TLC patterns of root extracts of samples collected in northern Sichuan Province were complex, suggesting that the five chemotypes are not distinct. Indeed, two of 38 samples were identified as H/C and H/N/V types, respectively [3]. In the present study, we analyzed the chemical composition of three samples collected in this area in detail (Table 1). Although these samples were previously assigned to H, N, and V types, respectively, many spots were detected on TLC. Seven new compounds (Fig. 1) were isolated and their structures were elucidated. Thirty-three known compounds were identified as well. The chemotypes of these samples were revised (Table 1).

The three samples were analyzed by use of LC-MS and the total ion chromatograms (TICs) are shown in Fig. 2. The TICs of all samples were different from each other. The compounds in each sample were isolated by EtOAc extraction from dried roots followed by spectroscopic analyses. The structure of the new compounds, **1**–**7**, were determined as follows.

2. Results and discussion

Compound **1** had a molecular formula of $C_{15}H_{20}O_4$ as determined from HRMS and ^{13}C NMR data (Table 2). The IR absorption bands at 3402, 1769, and 1722 cm^{-1} suggested the presence of hydroxy and carbonyl groups. The ^{1}H and ^{13}C NMR spectra (Table 2) displayed two sets of signals suggesting the presence of two isomeric compounds, which consisted of the signals of three methyls (δ_H 0.57, 0.58; 1.06, 1.07; 1.35, 1.36), formyl groups (δ_H 9.24,

* Corresponding author.
E-mail address: saiyoshi@nagasaki-u.ac.jp (Y. Saito).

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Table 1
Growing localities and chemotypes of three samples.

Sample No.	Specimen No.	Location	Altitude (m)	Chemotype (ref. 3)	Chemotype (revised)
1[a]	2010–46	Maierma (Aba county)	3600	H	H/C
2[b]	2010–52	A border of Ruoergai & Hongyuan counties	3600	N	N
3[c]	2010–63	A border of Songpan & Hongyuan counties	3600	V	N

[a] Sample 31 of ref. 3.
[b] Sample 34 of ref. 3.
[c] Sample 35 of ref. 3.

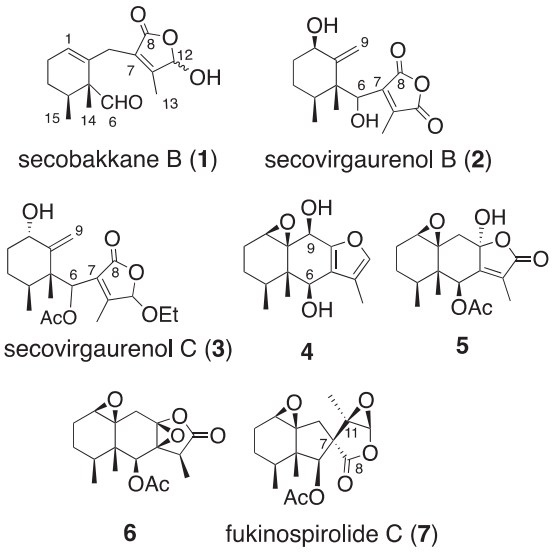

Fig. 1. New compounds (**1**–**7**) isolated from *L. virgaurea* samples 1–3.

9.27; δ_C 203.6, 203.8), olefins (δ_H 5.44, 5.48; δ_C 127.0, 127.2, 133.0, 133.1, 156.8), and oxymethine groups (δ_H 5.11, 5.22; δ_C 97.7, 97.8). The HMBC correlations from H_3-15 (δ_H 0.57, 0.58) to C-3 (δ_C 25.3, 25.4), C-4 (δ_C 32.1), and C-5 (δ_C 55.4), from H_3-14 (δ_H 1.06, 1.07) to C-4 (δ_C 32.1), C-5 (δ_C 55.4), C-6 (δ_C 203.6, 203.8), and C-10 (δ_C 133.0, 133.1), from H_3-13 (δ_H 1.35, 1.36) to C-7 (δ_C 127.0), C-11 (δ_C 156.8), and C-12 (δ_C 97.7, 97.8), and from H_2-9 (δ_H 2.52, 2.67) to C-8 (δ_C 171.0), C-10 (δ_C 133.0, 133.1), and C-11 (δ_C 156.8) as well as COSY correlations H-1/H_2-2/H_2-3 indicated a seco-bakkane skeleton as depicted in Fig. 3. Two sets of signals were attributed to the presence of acetal isomers at C-12 (*ca.* 1:1). NOE between a formyl proton (H-6) and H-4α and H_3-15 and between H_3-14 and H_3-15 showed that the formyl group was in the quasi-equatorial orientation in the cyclohexene ring as shown in Fig. 3. Therefore, the two methyl groups should be in the β-orientation. This skeleton presumably derived from a precursor such as fukinospirolide A (**33**) [3] by opening an epoxide followed by bond fission between C-6 and C-7. The 1,10-dihydro derivative **1a** was isolated from *L. lamarum* [7]. Compound **1a** is now named secobakkane A (**1a**) [7] and compound **1** secobakkane B.

The molecular formula of compound **2**, $C_{15}H_{20}O_5$ was deduced from the pseudo-molecular ion peak at *m/z* 263.1286 corresponding to $C_{15}H_{19}O_4$ $[M\text{-}H_2O + H]^+$ in HRCIMS and ^{13}C NMR data (Table 2). The IR absorption bands at 3400, 1828, and 1759 cm^{-1} suggested the presence of hydroxy and anhydride groups. The ^1H and ^{13}C NMR data (Table 2) showed the presence of three methyls (δ_H 0.61.0.67, 1.66), an exomethylene group (δ_H 4.46, 5.23; δ_C 109.9, 151.7), and two oxymethine groups (δ_H 4.54, 4.57; δ_C 69.7, 69.9). The HMBC spectrum indicated correlations between H_3-15 and C-3, C-4, and C-5, between H_3-14 and C-4, C-5, C-6, and C-10, between H_3-13 and C-7, C-11, and C-12, between H-6 and C-7, C-8, and C-11, and between H_2-9 and C-1, C-5, and C-10. These observations and COSY correlations H-1/H_2-2/H_2-3 suggested a seco-eremophilane skeleton as shown in Fig. 4. The stereochemistry was determined by the NOESY. NOE correlations between H_3-15 and H-2β and H_3-14, as well as between H_3-14 and H-4α, indicated that H_3-15 adopted β-axial orientation and H_3-14 β-equatorial, as shown in Fig. 4. The

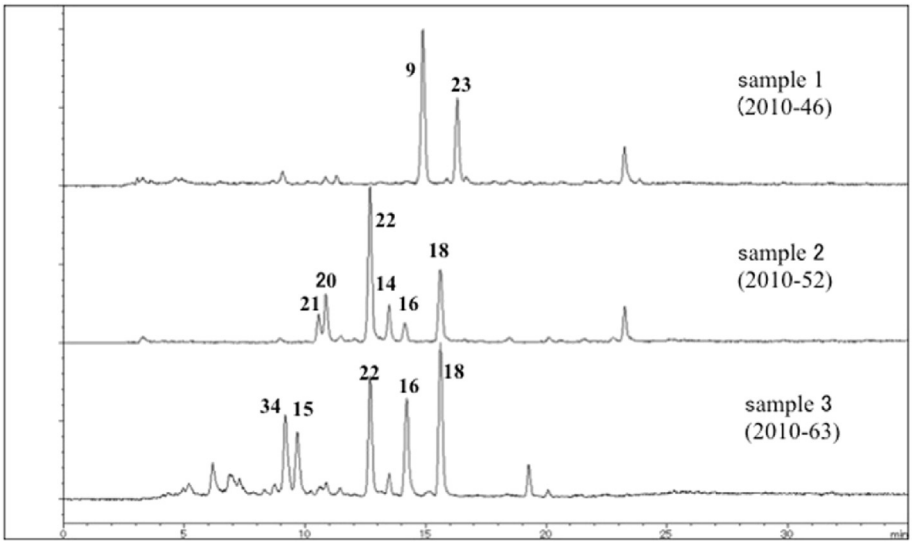

Fig. 2. LC profiles (total ion chromatograms) for samples 1–3.

Table 2
^{1}H and ^{13}C NMR data for secobakkane B (**1**), secovirgaurenol B (**2**), and secovirgaurenol C (**3**).

No.	secobakkane B (1)		secovirgaurenol B (2)		secovirgaurenol C (3)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.48, 5.44 (br s)	127.2[a]	4.54 (dd, 12.0, 4.0)	69.7	4.49 (t, 2.7)	73.1
2a	1.72 (m)	25.2[b]	1.74 (m)	30.9	2.00 (dq, 13.9, 2.7)	28.4
2b	1.72 (m)		1.39 (qd, 12.0, 4.0)		1.70 (m)	
3a	1.20 (m)	25.4, 25.3[b]	1.58 (m)	27.2	2.55 (tt, 13.4, 2.7)	24.1
3b	1.08 (m)		1.16 (m)		1.16 (m)	
4	1.57 (m)	32.1	2.08 (m)	33.0	2.20 (m)	34.1
5	–	55.4	–	51.0	–	47.5
6	9.27, 9.24 (s)	203.8, 203.6	4.57 (br s)	69.9	7.21 (s)	72.8
7	–	127.0[a]	–	144.0[c]	–	129.6
8	–	171.0	–	165.8[d]	–	172.4
9a	2.52 (m)	26.9, 26.8	5.23 (br s)	109.9	4.70 (br s)	117.3
9b	2.67 (m)		4.46 (br s)		5.06 (s)	
10	–	133.1, 133.0	–	151.7	–	150.4
11	–	156.8	–	143.3[c]	–	160.2
12	5.22, 5.11 (s)	97.8, 97.7	–	165.2[d]	4.95 (s)	102.5
13	1.36, 1.35 (s)	11.1, 11.0	1.66 (s)	10.9	1.67 (s)	13.2
14	1.07, 1.06 (s)	13.4, 13.3	0.61 (s)	17.2	0.89 (s)	18.6
15	0.58 (d, 6.3), 0.57 (d, 6.9)	15.9	0.67 (d, 7.3)	15.7	0.82 (d, 7.1)	16.3
1′	–	–	–	–	–	168.1
2′	–	–	–	–	1.52 (s)	19.7
1″	–	–	–	–	3.37 (qd, 7.1, 9.3)	64.3
					3.18 (qd, 7.1, 9.3)	
2″	–	–	–	–	0.88 (t, 7.1)	15.1

[a,b,c,d] Assignments may be interchanged.

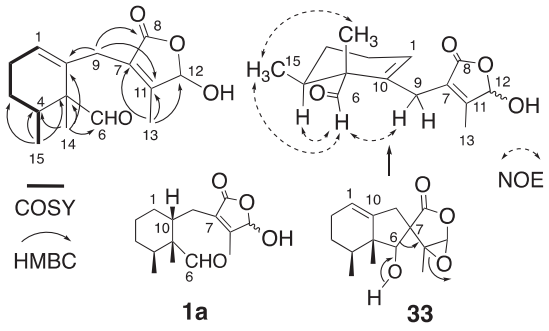

Fig. 3. Major 2D correlations detected for compound **1** and the structure of related compounds.

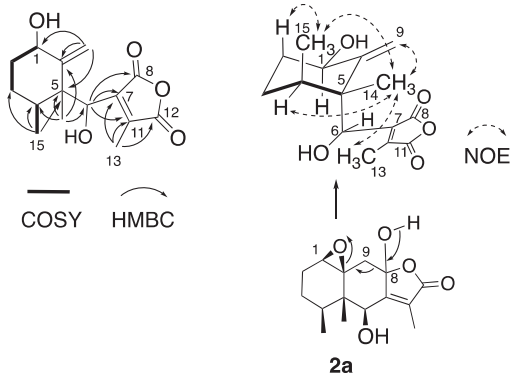

Fig. 4. Major 2D correlations detected for compound **2** and the structure of its plausible biosynthetic precursor **2a**.

configuration of the hydroxy group at C-1 was determined to be β, because the coupling pattern of H-1 was a doublet of doublets with $J = 12.0$ and 4.0 Hz. The configuration of the hydroxy group at C-6 was not determined, but it would be most probably β (S^*) as found in most eremophilanes. It was also deduced that compound **2** was biosynthetically derived from such as compound **2a** through opening an epoxide followed by C-8/C-9 bond fission forming a carbonyl group by elimination of a hydroxy proton, accounting for both configurations at C-1 and C-6.

Compound **3**, $C_{19}H_{28}O_6$ (by HRMS), showed 19 signals in its ^{13}C NMR spectrum (Table 2), in which five methyls, four methylenes, four methines, and six quaternary carbons were detected. Protons at δ 4.70 and 5.06 were assigned to an exomethylene, and an acetoxy (δ_H 1.52) and ethoxy groups were also detected (Table 2). The HMBC and COSY correlations shown in Fig. 5 indicated that this was also a C-8/C-9 cleaved eremophilane. The difference compared with compound **2** was that there was an acetyl group in compound **3** and an anhydride moiety was reduced to an ethoxy lactol at C-12. The configuration of a hydroxy group at C-1 was determined to be α, because H-1 resonated at δ 4.49 as a triplet with $J = 2.7$ Hz indicating the equatorial nature. The conformation of the methylenecyclohexane ring was almost the same as that of compound **2**. The structure of compound **3** was established as depicted in Fig. 5. Compound **3** was an acetylated derivative of compound **3a**, which was previously isolated from *L. virgaurea* [3]. Compound **3a** is now named secovirgaurenol A, compound **2** secovirgaurenol B, and

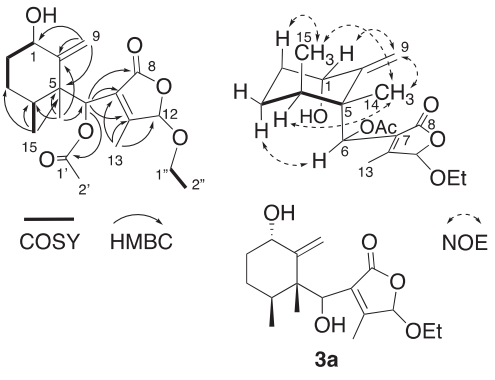

Fig. 5. Major 2D correlations detected for compound **3**.

compound **3** secovirgaurenol C. It is worth mentioning that the α-substituent at C-5 of all secovirgaurenols A, B, and C (**3a**, **2**, **3**) adopted α-axial position despite their large sizes. This unusual conformation of six-membered ring in secovirgaurenols would be due to allylic strain.

The ^{1}H NMR spectrum (Table 3) of compound **4**, $C_{15}H_{20}O_4$ (by HRMS), showed the signals of three methyls, a furan, and three oxymethine groups. Analyses of HMBC and COSY spectra suggested a furanoeremophilane skeleton (Fig. 6). Two hydroxy groups were indicated to be at C-6 and C-9 positions using the HMBC spectrum. Two carbons at δ_C 63.4 and 66.0 were attributed to an epoxide, which was assigned between C-1 and C-10 using the HMBC spectrum (Fig. 6). NOE between: H-6 and H-4α; H-6 and H-3α; H_3-14 and H_3-15; H-1 and H-2α; and H-1 and H-9α indicated that the stereochemistry should be as depicted in Fig. 6.

The molecular formula of compound **5** was determined as $C_{17}H_{22}O_6$ from HRMS and ^{13}C NMR data (Table 3). The IR spectrum showed absorption bands of a hydroxy and an ester. The ^{1}H NMR spectrum (Table 3) indicated the presence of four methyl (including an acetyl group) and two oxymethine protons. The ^{13}C NMR spectrum (Table 3) showed the presence of two carbonyl, two olefinic, and four carbons bearing oxygen functions. HMBC and COSY spectra suggested an eremophilenolide skeleton with an acetoxy group at C-6 and an epoxide at C-1 and C-10 positions (Fig. 7). NOE between H-6 and H-4α and between H_3-14 and H-9β indicated the stereochemistry as shown in Fig. 7. The hydroxy group at C-8 should be α-oriented, because if this had been β-oriented, NOE correlations shown in Fig. 7 would not be observed [8].

The molecular formula of compound **6** was determined to be

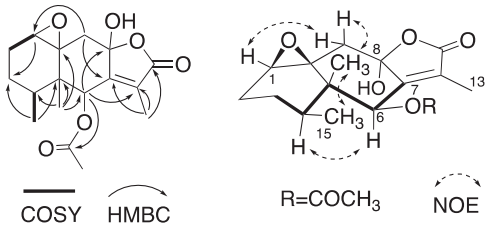

Fig. 7. Major 2D correlations detected for compound **5**.

$C_{17}H_{22}O_6$ from HRMS and ^{13}C NMR data (Table 4). The ^{1}H and ^{13}C NMR spectra (Table 4) indicated the presence of four methyls, three methylenes, four methines, and six quaternary carbon atoms. The IR absorption at 1807 cm^{-1} suggested the presence of an epoxy- or enol-lactone [3]. The ^{13}C NMR signals assigned to C-7 and C-8 were observed at δ_C 64.5 and 85.7, respectively; therefore, this compound was determined as an epoxy lactone of eremophilanolide, supported by the analyses of 2D NMR spectra (Fig. 8). Another epoxide and an acetoxy group were assigned from HMBC at C-1/C-10 and at C-6, respectively. NOE between: H-6 and H-11α; H-6 and H-4α; and H_3-14 and H-9β were observed and indicated the stereochemistry as shown in Fig. 8.

Compound **7** had a molecular formula $C_{17}H_{22}O_6$ determined from HRMS and ^{13}C NMR data (Table 4). The ^{1}H NMR spectrum (Table 4) showed signals of three singlet methyl groups, one of which was an acetoxy group, a doublet methyl, and three oxymethine protons. The ^{13}C NMR spectrum (Table 4) showed signals of four methyl, three methylene, four methine, and six quaternary

Table 3
^{1}H and ^{13}C NMR data for compounds **4** and **5**.

No.	4		5	
	δ_H	δ_C	δ_H	δ_C
1	2.50 (d, 4.9)	63.4	2.89 (d, 3.6)	62.2
2a	1.19 (m)	20.1	1.45 (m)	20.3
2b	1.71 (m)		1.74 (m)	
3a	1.22 (m)	24.2	1.47 (m)	24.0
3b	1.02 (m)		1.06 (m)	
4	1.73 (m)	32.8	1.44 (m)	32.6
5	–	41.1	–	43.3
6	4.53 (d, 10.4)	69.3	5.98 (q, 1.7)	73.5
7	–	123.1	–	154.7
8	–	146.8	–	101.0
9a	3.78 (s)	68.7	1.65 (d, 13.5)	43.5
9b			2.12 (d, 13.5)	
10	–	66.0	–	60.8
11	–	120.4	–	124.6
12	6.92 (s)	140.9	–	170.5
13	1.98 (s)	9.2	1.71 (d, 1.7)	8.1
14	1.24 (s)	16.9	0.86 (s)	14.4
15	1.07 (d, 7.1)	15.7	0.97 (d, 7.4)	16.0
1′	–	–	–	169.9
2′	–	–	1.56 (s)	20.0
6-OH	1.11 (d, 10.4)	–	–	–
9-OH	2.18 (s)	–	–	–

Table 4
^{1}H and ^{13}C NMR data of compounds **6** and **7**.

No.	6		7	
	δ_H	δ_C	δ_H	δ_C
1	2.47 (d, 4.9)	63.0	3.02 (br s)	62.5
2a	1.39 (m)	19.9	1.81 (m)	25.9
2b	1.71 (m)		1.85 (m)	
3a	1.53 (m)	23.6	0.85 (m)	23.7
3b	1.06 (m)		1.22 (dtd, 13.7, 12.0, 5.6)	
4	1.33 (m)	33.4	1.67 (m)	36.8
5	–	40.1	–	45.2
6	5.57 (s)	70.9	5.57 (s)	82.1
7	–	64.5	–	55.9
8	–	85.7	–	177.3
9a	1.54 (d, 15.6)	30.8	1.13 (d, 13.4)	36.3
9b	2.69 (d, 15.6)		2.24 (d, 13.4)	
10	–	60.7	–	67.7
11	2.41 (q, 7.1)	42.6	–	61.7
12	–	175.1	4.41 (s)	82.2
13	1.17 (d, 7.1)	11.4	0.89 (s)	14.7
14	1.29 (s)	15.3	0.92 (s)	11.5
15	0.95 (d, 7.3)	15.2	0.82 (d, 6.8)	16.2
1′	–	170.3	–	167.9
2′	1.54 (s)	19.9	1.66 (s)	20.2

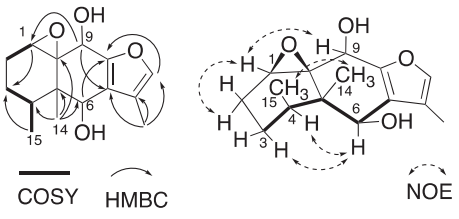

Fig. 6. Major 2D correlations detected for compound **4**.

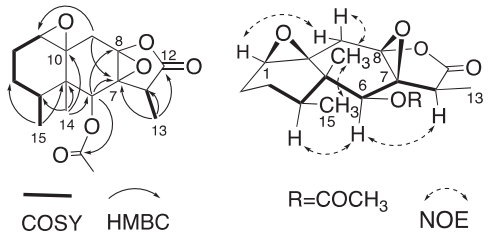

Fig. 8. Major 2D correlations detected for compound **6**.

carbon atoms. The HMBC spectrum indicated the correlations between: H_3-15 and C-3, C-4, and C-5; H_3-14 and C-4, C-5, C-6, and C-10; H_3-13 and C-7, C-11, and C-12; H-12 and C-8; H-6 and C-7, C-8, C-9, and C-1'; H-9 and C-1; and H-1 and C-3 (Fig. 9). From these results as well as COSY correlations H-1/H_2-2 indicated a bakkane skeleton with an acetoxy group at C-6, and epoxides at C-1/C-10 and C-11/C-12. NOE between H_3-13 and H-9β indicated that C-11 was β-orientation (upward drawing) [1] and the epoxide at C-11/C-12 was β-configuration (Fig. 9). The conformation was suggested by NOE correlations as shown in Fig. 9 and hence, the epoxide at C-1/C-10 was also determined to be β-orientation. This compound was named fukinospirolide C.

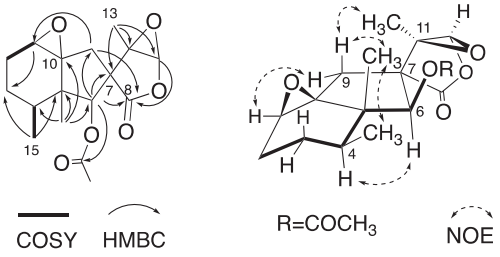

Fig. 9. Major 2D correlations detected for compound **7**.

Other compounds, **8**–**40** (Fig. 10), were identified by comparing their spectroscopic data with those of compounds isolated by us, and their sources are listed in Table 5. Compound **40**, friedelin, was isolated from *Ligularia* for the first time.

Major peaks detected in the LCMS (Fig. 2) were assigned by the measurement of isolated compounds (Fig. 10). Two major peaks in sample 1 (t_R = 14.9 and 16.4 min) were assigned as 6-hydroxyeuryopsin (**9**) and cacalol (**23**), respectively. Two major peaks in samples 2 and 3 (t_R = 12.7 and 15.7 min) were assigned as 1,10-epoxyadenostylone (**22**) and neoadenostylone (**18**), respectively.

We previously determined chemotypes by Ehrlich's test on TLC [3], which is a useful method to detect furanoeremophilanes quickly [9]. However, it is better to discuss chemotype from LCMS data and the structures of isolated compounds. The chromatogram

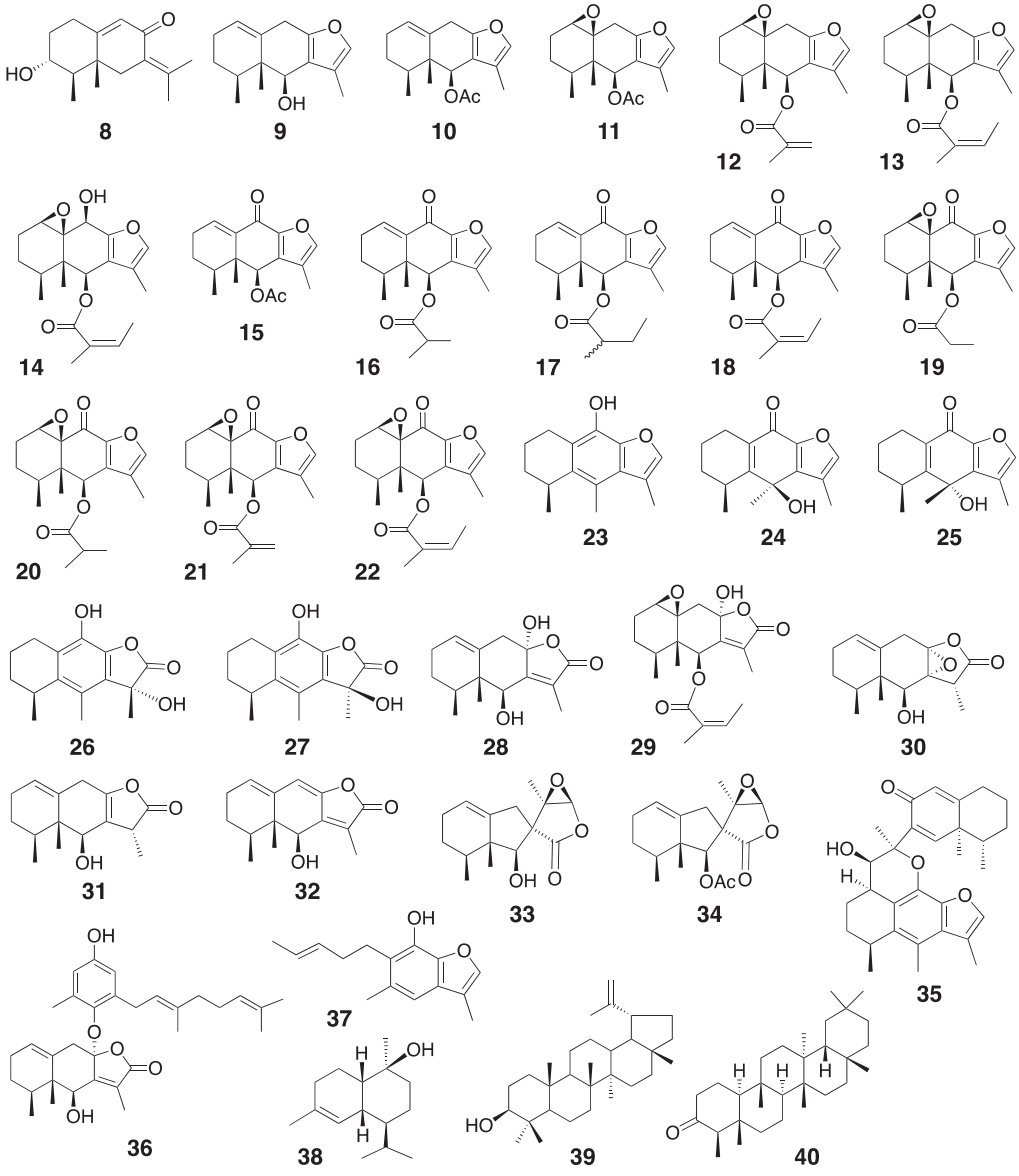

Fig. 10. The structures of known compounds **8**–**40**.

Table 5
Isolated compounds.[a]

No.	Eremophilanes				Others
	9-Non-oxygenated[b]	9-Oxygenated[c]	Cacalols[d,e]	Simple	
1	**9**, **28**, **30**, **31**, **32**, **36**	**4***, **16**, **17**, **20**	**23**, **24**, **25**, **26**, **27**, **35**	**8**	**1***, **2***, **33**, **37**, **38**, **39**
2	**12**, **13**	**14**, **16**, **18**, **19**, **20**, **21**, **22**			**39**
3	**5***, **6***, **10**, **11**, **13**, **29**	**14**, **15**, **18**, **22**			**3***, **7***, **34**, **40**

[a] Asterisks and underlines denote new compounds and major constituents, respectively.
[b] Compounds characteristic of H type. The most typical compound is 6-hydroxyeuryopsin (**9**).
[c] Compounds characteristic of N type. The most typical compound is neoadenostylone (**18**).
[d] Compounds characteristic of C type. The most typical compound is cacalol (**23**).
[e] Methyl-migrated eremophilanes.

of sample 1 showed the presence of two major peaks of **9** and **23** (a peak at $t_R = 23$ min is an impurity), characteristic of H and C type compounds, respectively, and therefore, sample 1 was assigned as H/C type. Sample 2 showed two major compounds **18** and **22**, both of which were characteristic of N type compounds (9-oxygenated derivatives). The TIC of sample 3 was complex but major peaks were similar to sample 2 (**18** and **22**). In addition, two more 9-oxo derivatives **15** and **16** were detected. Thus, sample 3 was judged to be N type.

Compounds belonging to H and N types are similar in structure (both are furanoeremophilanes). The only difference is the oxidation level at C-9; N type has an oxygen function at C-9, but H type has no such functional group [3]. Other substituents, including the presence of (i) either 1(10)-enes or 1,10-epoxides, (ii) oxygen functionality at C-6, and (iii) no functionality at C-3, are common in these two chemotypes. Furanoeremophilan-9-ones are sometimes isolated from *Ligularia* together with 9-non-oxygenated derivatives [10]. In the present study, both H and N type compounds were isolated from all three samples (Table 5). These data suggested that H and N types are continuous. Previously, we reported the presence of an H/N/V type [3], from which typical compounds of all H, N, and V types were isolated. In sample 3, although V type compounds were not isolated, typical yellow spots of V type were detected on TLC (thus this sample was previously assigned to V type). These observations indicate that H and N types are also continuous with V type. Allylic oxidation from H type [1(10)-ene] to either N type [1(10)-en-9-one] or V type [1(10)-en-2-one] is a plausible biosynthetic pathway. Sample 1 was the second example of H/C type, suggesting that C type is also continuous with H type. Cacalol (typical of C type compounds) may be generated from furanoeremophilan-9-one derivatives [11,12]. These results indicate that four chemotypes, H, N, V, and C, are continuous and now under reticulated evolution in the northern Sichuan area.

3. Conclusion

Seven new compounds, **1**–**7**, were isolated from three samples of *L. virgaurea* collected in China. The chemotypes of samples 1 and 3 were revised to a mixture of H and C types and N type, respectively. Three new compounds were seco derivatives, two secoeremophilanes and one secobakkane. Secobakkane B (**1**) was presumably derived from a bakkane type compound through bond fission between C-6 and C-7, which was the second example of C-6/C-7 secobakkane [3].

4. Experimental

4.1. General

Specific rotations and circular dichroism (CD) spectra were measured on a JASCO DIP-1030 and a JASCO J-725 auto recording polarimeter; IR spectra, on a Shimadzu FT/IR-8400S spectrophotometer with the diffuse reflectance method; ^{1}H and ^{13}C NMR spectra, on a Varian 500-MR (500 MHz and 125 MHz, respectively) spectrometer (in C_6D_6). Mass spectra, including high-resolution spectra, were recorded on a JEOL JMS-700 MStation. Chemcopak Nucleosil 50-5 (4.6 × 250 mm) (a JASCO pump system) was used for HPLC with a solvent system of hexane-ethyl acetate. LC-MS was measured on an Agilent 1100 series LC/MSD mass spectrometer with 5C18-MS-II using gradient system (MeOH/H_2O) using EtOH extracts of samples (see ref. 5 for the details). Silica gel 60 (70–230 mesh, Fuji Silysia) was used for column chromatography. Silica gel 60 F_{254} plates (Merck) were used for TLC.

4.2. Plant materials

Samples were collected in August 2010 at the locations shown in Table 1 and were identified by X. G., one of the authors. The voucher specimen numbers of samples 1–3 are 2010–46, 2010–52, and 2010–63, respectively (Kunming Institute of Botany).

4.3. Isolation

The root of each sample was dried, cut into pieces, and extracted with EtOAc to give the extracts. The compounds were separated by silica-gel column chromatography (hexane-EtOAc) and HPLC (Nucleosil 50-5 and TSK-GEL G1000H_{HR}; hexane-EtOAc) to isolate each compound.

The roots of sample 1 (2010–46) (dry weight 21.7 g) afforded an extract (2158.9 mg). This extract was separated to isolate secobakkane B (**1**) (4.3 mg), secovirgaurenol B (**2**) (1.1 mg), **4** (1.2 mg), **8** [13] (1.0 mg), 6-hydroxyeuryopsin (**9**) [14] (354.8 mg), **16** [10a] (3.8 mg), **17** [15] (0.8 mg), **20** [15] (7.4 mg), cacalol (**23**) [12,16] (96.9 mg), epicacalone (**24**) [17] (14.0 mg), cacalone (**25**) [17] (14.8 mg), hydroxycacalolide (**26**) [17] (0.3 mg), hydroxyepicacalolide (**27**) [17] (1.2 mg), **28** [3] (3.4 mg), **30** [7] (9.3 mg), **31** [18] (10.6 mg), **32** [3] (6.1 mg), fukinospirolide A (**33**) [3] (7.7 mg), virgaurin C (**35**) [3] (3.1 mg), **36** [3] (5.7 mg), **37** [19] (12.9 mg), **38** [20] (9.3 mg), and lupeol (**39**) [21] (2.3 mg).

The roots of sample 2 (2010–52) (dry weight 4.6 g) afforded an extract (406.5 mg). This extract was separated to isolate **12** [22] (1.9 mg), **13** [10a] (2.4 mg), **14** [23] (87.7 mg), **16** (6.2 mg), neoadenostylone (**18**) [24] (42.1 mg), **19** [15] (1.4 mg), **20** (20.4 mg), **21** [15] (6.1 mg), **22** [15] (17.9 mg), and **39** (1.6 mg).

The roots of sample 3 (2010–63) (dry weight 4.6 g) afforded an extract (266.8 mg). This extract was separated to isolate **3** (1.3 mg), **5** (4.3 mg), **6** (1.8 mg), **7** (1.2 mg), **10** [25] (5.4 mg), **11** [10a] (2.7 mg), **13** (1.6 mg), **14** (0.8 mg), **15** [26] (3.6 mg), **18** (25.3 mg), **22** (15.7 mg), **29** [27] (0.8 mg), fukinospirolide B (**34**) [3] (2.5 mg), and friedelin (**40**) (2.3 mg).

4.4. Compound data

4.4.1. Secobakkane B (**1**)

Oil; $[\alpha]_D^{18}$ +42.9 (*c* 0.12, EtOH); IR (KBr) 3402, 1769, 1722 cm^{-1}; MS (CI) *m/z* 265 $[M+H]^+$, 247 (100), 219, 205, 109; HRMS (CI) Obs. *m/z* 265.1445 (Calcd for $C_{15}H_{21}O_4$ 265.1440); ^1H and ^{13}C NMR: see Table 2.

4.4.2. Secovirgaurenol B (**2**)

Oil; $[\alpha]_D^{23}$ −12.9 (*c* 0.08, EtOH); IR (KBr) 3400, 1828, 1759, 1645 cm^{-1}; MS (CI) *m/z* 263 $[M\text{-}H_2O+H]^+$, 245, 217, 139, 121 (100); HRMS (CI) Obs. *m/z* 263.1286 $[M\text{-}H_2O+H]^+$ (Calcd for $C_{15}H_{19}O_4$ 263.1283); ^1H and ^{13}C NMR: see Table 2.

4.4.3. Secovirgaurenol C (**3**)

Oil; $[\alpha]_D^{22}$ +21.5 (*c* 0.13, EtOH); IR (KBr) 3425, 1741, 1231 cm^{-1}; MS (FAB) *m/z* 375 $[M+Na]^+$, 353 $[M+H]^+$, 335, 229, 173 (100); HRMS (FAB) Obs. *m/z* 375.1788 $[M+Na]^+$ Calcd for ($C_{19}H_{28}O_6Na$ 375.1784); ^1H and ^{13}C NMR: see Table 2.

4.4.4. Compound **4**

Oil; $[\alpha]_D^{16}$ +35.5 (*c* 0.08, EtOH); IR (KBr) 3458 cm^{-1}; MS (CI) *m/z* 265 $[M+H]^+$, 247 (100), 229, 219, 201, 191, 138, 123; HRMS (CI) Obs. *m/z* 265.1435 $[M+H]^+$ Calcd for ($C_{15}H_{21}O_4$ 265.1440); ^1H and ^{13}C NMR: see Table 3.

4.4.5. Compound **5**

Oil; $[\alpha]_D^{23}$ −28.5 (*c* 0.4, EtOH); IR (KBr) 3310, 1766, 1745 cm^{-1}; MS (CI) *m/z* 323 $[M+H]^+$, 305, 263, 245 (100), 217; HRMS (CI) Obs. *m/z* 323.1496 (Calcd for $C_{17}H_{23}O_6$ 323.1495); CD (EtOH) θ (nm): −100 (219), −40 (230), −800 (245); ^1H and ^{13}C NMR: see Table 3.

4.4.6. Compound **6**

Oil; $[\alpha]_D^{22}$ −14.5 (*c* 0.18, EtOH); IR (KBr) 1807, 1745, 1232 cm^{-1}; MS (CI) *m/z* 323 $[M+H]^+$, 281, 263, 245, 235 (100), 217, 207; HRMS (CI) Obs. *m/z* 323.1482 (Calcd for $C_{17}H_{23}O_6$ 323.1495); CD (EtOH) θ (nm): +4500 (220), −50 (259), +90 (324); ^1H and ^{13}C NMR: see Table 4.

4.4.7. Fukinospirolide C (7)

Oil; $[\alpha]_D^{21}$ +19.6 (*c* 0.12, EtOH); IR (KBr) 1794, 1778, 1745, 1227 cm^{-1}; MS (CI) *m/z* 323 $[M+H]^+$, 281, 263 (100), 245, 235, 217; HRMS (CI) Obs. *m/z* 323.1485 (Calcd. $C_{17}H_{23}O_6$ 323.1495); CD (EtOH) θ (nm): +2400 (225), −230 (257), +240 (281), −140 (362); ^1H and ^{13}C NMR: see Table 4.

Conflicts of interest

The authors declare no conflict of interest.

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