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Structures of six new compounds from *Ligularia brassicoides*

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ABSTRACT

Four new eremophilane type sesquiterpenoids, a new bakkane type sesquiterpenoid, and a new secoeremophilane type sesquiterpenoid were isolated from *Ligularia brassicoides* Hand.-Mass. (Asteraceae) collected in China, and were an epoxy lactone derived from 6-acyloxyeuryopsin **1**, 6-acyloxy-9-oxoeuryopsin **2**, 6-acyloxy-9-oxofuranoeremophilane **3**, 6-acyloxyeuryopsin **4**, 6-acyloxy bakkane type epoxy lactone **5**, and secovirgaurenol 6-*O*-(2′-hydroxymethyl)acrylate (**6**), respectively. All but one had a 2′-hydroxymethylacryloyloxy group at C-6. This is the first study to describe the chemical constituents of this species.

1. Introduction

The genus *Ligularia* (Asteraceae) is highly diversified in the Hengduan Mountains of China, forming a complex with related genera such as *Cremanthodium* and *Parasenecio*.[1] We previously examined the diversity of *Ligularia* species in that area.[2–11] We found that intra-specific diversity was present in many species with various modes, implying that the mechanism(s) underlying the generation of chemical diversity may be complex.[11]

In the present study, we focused on *Ligularia brassicoides* Hand.-Mazz., which is taxonomically close to *Ligularia dictyoneura* (Franch.) Hand.-Mazz. The two species have leathery leaves as their characteristic morphology.[12] *L. brassicoides* grows on the grassy slopes of western Sichuan, while *L. dictyoneura* grows on the stream banks, forest understories, scrub, and grassy slopes of northwest Yunnan and southwest Sichuan.[12] We previously reported that the chemical composition of *L. dictyoneura* was highly diverse, producing furanoeremophilanes and/or eremophilan-8-ones.[9,11] In the course of our continuing research in this area, we collected two samples of *L. brassicoides* in the summer of 2007. Eight compounds, six of which were new, were isolated (Fig. 1). All the new compounds had a 2-hydroxymethyl-acryloyloxy group at C-6, except for one, which had an epoxide instead of a hydroxymethyl group in an ester moiety. All were supposed to be derived from an eremophilane compound and the results were similar to those of type 6 of *L. dictyoneura*[9] and type H of *Ligularia virgaurea var. virgaurea* (Maxim.) Mattf.[2b] In the present study, we described the isolation and structure elucidation of six new compounds. To the best of our knowledge, this is the first study to report the chemical constituents of this species.

2. Results and discussion

The roots of two samples were cut into pieces and extracted with EtOAc. The oily residue was separated by silica gel column chromatography and HPLC to afford eight compounds, six of which were new. The structures of six new compounds were elucidated as follows.

Compound **1** exhibited a quasi-molecular ion peak at *m*/*z* 349 and its molecular formula was determined to be $C_{19}H_{24}O_6$ by HRMS and ^{13}C NMR data. Its IR spectrum revealed the presence of

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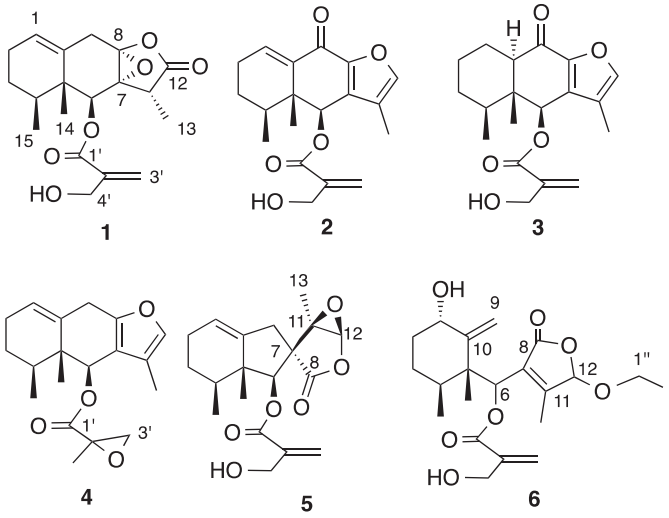

Fig. 1. New compounds isolated in this study.

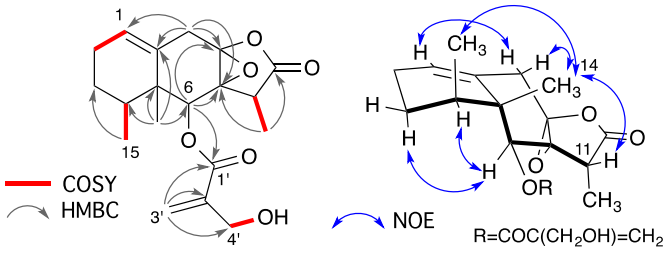

Fig. 2. Selected 2D correlations of compound **1**.

a hydroxy (3600–3400 cm^{-1}), an enol- or epoxy-lactone (1807 cm^{-1}),[2b,c,5,6] and an ester (1718 cm^{-1}) group as well as a double bond (1637 cm^{-1}). ^1H NMR showed the presence of tri-substituted alkene (δ 5.28), exomethylene (δ 5.53 and 6.00), oxymethine (δ 5.49), and oxymethylene (δ 4.00, 2H) groups (Table 1). ^{13}C NMR data indicated the presence of three methyl, five methylene, four methine, and seven quaternary carbon atoms. Because the degree of unsaturation was eight and the presence of two carbonyl groups and two double bonds was indicated, this compound appeared to be tetracyclic. An eremophilane skeleton was suggested by COSY correlations of H-1/H_2-2, H-4/H_3-15, H-11/H_3-13, and H_2-4′/*OH* and HMBC correlations between H_3-15 and C-3, between H_3-14 and C-4, C-5, C-6, and C-10, between H_3-13 and C-7 and C-12, and other correlations (Fig. 2). C-7 and C-8 were inferred to be epoxides from the chemical shifts (δ_C 65.4 and 87.1) (Table 2). The acyl group was suggested to be a 2′-hydroxymethylacryloyl group because a correlation was detected between H_2-3′ (δ_H 5.53 and 6.00) and C-1′ (δ_C 164.9), C-2′ (δ_C 139.6), and C-4′ (δ_C 61.8). The position of this acyl group was determined to be at C-6 by the HMBC correlation between H-6 and C-1′. Therefore, the structure was established to be 6-(2′-hydroxymethylacryloyloxy)-7,8-epoxyeremophil-1(10)-en-(12,8)-olide. Stereochemistry was

Table 2
^{13}C NMR data of compounds **1**–**6** (δ_C, 100 MHz, in C_6D_6)

Position	1	2	3	4	5	6
1	129.3	137.5	21.1	124.8	122.0	73.1
2	21.4	25.1	25.2	21.7	25.6	28.4
3	25.7	28.0	32.4	26.6	27.2	24.0
4	32.1	38.0	42.2	32.6	38.6	34.3
5	41.6	46.4	49.4	42.8	46.8	47.7
6	72.3	75.0	76.1	74.0	83.8	73.1
7	65.4	134.6	133.7	117.6	55.6	129.2
8	87.1	147.9[a]	147.6	151.5	178.0	172.4
9	31.4	175.5	185.5	31.5	37.7	117.6
10	130.3	141.4	54.9	135.0	140.1	150.1
11	40.6	120.9	120.7	120.3	61.3	160.6
12	175.3	145.6	144.8	138.9	81.1	102.6
13	11.0	8.4	8.6	8.9	14.0	13.4
14	16.6	15.6	7.7	16.8	14.1	18.9
15	14.9	17.6	18.0	15.3	16.5	16.3
1′	164.9	165.7	166.0	170.9	164.4	164.1
2′	139.6	140.1	140.3	53.8	140.7	140.0
3′	125.8	125.7	125.7	52.5	124.4	124.6
4′	61.8	61.9	61.9	17.7	62.2	62.0
1″	—	—	—	—	—	64.1
2″	—	—	—	—	—	14.9

[a] Not detected in the ^{13}C NMR; deduced from HMBC correlation.

Table 1
^1H NMR data of compounds **1**–**6** (δ_H (J in Hz), 400 MHz, in C_6D_6)

Position	1	2	3	4	5	6
1	5.28, m	7.01, dd (5.1, 3.1)	2.24, br d (15.1)	5.29, m	5.31, m	4.46, t (2.9)
	—	—	1.37, m	—	—	—
2	1.63, m	1.70, m	1.53, m	1.79, m	1.90, m	1.94, br dq (13.5, 2.9)
	1.52, m	—	0.93, m	1.61, m	1.81, m	1.67, m
3	1.63, m	1.07, m	1.02, m	1.79, m	1.24, m	2.46, tt (13.5, 3.8)
	1.05, m	—	0.93, m	1.27, m	1.16, m	1.10, dq (13.5, 3.8)
4	1.52, m	1.70, m	1.37, m	1.79, m	1.55, dqd (12.1, 6.8, 3.3)	2.26, qt (7.0, 3.8)
6	5.49, s	6.30, s	6.25, s	6.34, dd (2.7, 1.3)	5.78, s	7.27, s
9	2.66, br d (14.6)	—	—	3.29, br d (17.0)	2.33, dtd (15.4, 3.7, 2.6)	5.06, d (1.6)
	2.52, d (14.6)	—	—	2.85, d (17.0)	1.97, dd (15.4, 0.8)	4.71, d (1.6)
10	—	—	1.70, dd (12.1, 3.5)	—	—	—
11	2.77, q (7.3)	—	—	—	—	—
12	—	6.69, q (1.0)	6.69, q (1.0)	6.87, br s	4.40, s	4.96, s
13	1.23, q (7.3)	1.58, d (1.0)	1.64, d (1.0)	1.88, d (1.2)	0.98, s	1.70, s
14	0.68, s	0.97, s	0.76, s	0.97, s	0.89, s	0.95, s
15	0.62, d (7.1)	0.76, d (6.7)	0.72, d (6.7)	0.80, d (7.2)	0.77, d (6.8)	0.81, d (7.0)
3′	6.00, dt (1.4, 1.2)	6.14, dt (1.4, 1.2)	6.14, dt (1.4, 1.2)	2.91, d (6.3)	6.16, dt (1.6, 1.4)	6.03, dt (1.4, 1.2)
	5.53, td (1.6, 1.4)	5.60, td (1.8, 1.4)	5.60, td (1.7, 1.4)	2.15, d (6.3)	5.59, dt (1.8, 1.6)	5.51, td (1.6, 1.4)
4′	4.00, m	4.15, m	4.16, s	1.40, s	4.22, m	4.02, m
	—	—	—	—	—	—
3′-OH	1.12, br s	—	—	—	1.37, br t (6.1)	—
12-OEt	—	—	—	—	—	3.34, dq (9.4, 7.0)
	—	—	—	—	—	3.18, dq (9.4, 7.0)
	—	—	—	—	—	0.86, t (7.0)

determined by NOESY. Since a correlation was detected between H_3-14 and H-9β, its conformation should be as shown in Fig. 2. The configuration of H-11 was established to be β due to the presence of NOE between H-11 and H_3-14. If the epoxide was β-oriented, the NOE between H-11 and H_3-14 should not be detected. Therefore, both the epoxide and the methyl group at C-11 should be α-oriented, which was also explained by the plausible biogenetic pathways and precedents.[2b] The configurations of the acyloxy group at C-6 and the methyl group at C-4 were determined to be β, because the NOE between H-4 and H-6 was observed.

The molecular formula of compound **2** was determined to be $C_{19}H_{22}O_5$ by HRMS and ^{13}C NMR data. ^1H NMR showed the presence of a furan (δ 6.69), trisubstituted alkene (δ 7.01), exomethylene (δ 5.60 and 6.14), oxymethine (δ 6.30), and oxymethylene (δ 4.15, 2H) groups (Table 1). The 2D correlation shown in Fig. 3 indicated an eremophilane skeleton substituted with a 2′-hydroxymethylacryloyloxy moiety at C-6. The presence of a carbonyl group at C-9 was not directly indicated; however, the signal of C-7 was detected at δ_C 134.6, being shifted to a lower field than a normal furan (δ 115–120) (Table 2).[10,13] A similar phenomenon was described in previous studies.[10,13] The NOE between H-6 and H-4 indicated the configurations as shown in Fig. 3.

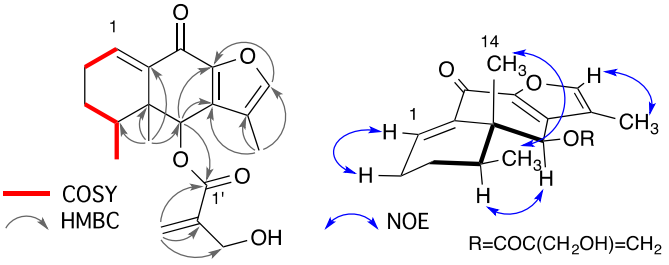

Fig. 3. Selected 2D correlations of compound **2**.

Compound **3** showed a quasi-molecular ion peak at *m*/*z* 333 and the molecular formula was determined to be $C_{19}H_{24}O_5$ by HRMS and ^{13}C NMR data. Spectroscopic data were similar to those of compound **2**, except that a double bond was absent in compound **3**. The eremophilane skeleton was easily confirmed by the 2D correlation shown in Fig. 4. The position of the carbonyl group was determined by the correlation between H-10 and C-9 as well as the chemical shift of C-7 (δ_C 133.7) as discussed above. The trans-relationship of H_3-14 and H-10 was determined by the NOEs between H-4 (δ 1.37) and H-10 (δ 1.70) and between H-6 (δ 6.25) and H-10. These results indicated that all three protons (H-4, 6, and 10) were α-oriented. Therefore, compound **3** was established as 6β-(2′-hydroxymethylacryloyloxy)-9-oxofuranoeremophilane.

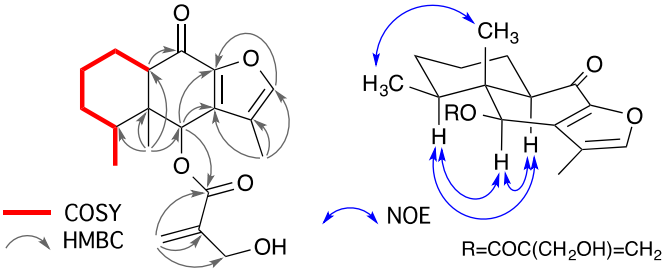

Fig. 4. Selected 2D correlations of compound **3**.

Compound **4** had a molecular formula of $C_{19}H_{24}O_4$ (by HRMS and ^{13}C NMR), one oxygen less than that of compound **3**. The spectroscopic difference between compounds **4** and **3** was that, in compound **4**, an exomethylene group was absent, the oxymethylene protons were shifted to a higher field (δ 2.15 and 2.91), and the carbonyl group was absent. The 2D correlation indicated an eremophilane skeleton, as shown in Fig. 5. The degree of unsaturation was eight and the presence of a furan (δ_C 117.6, 120.3, 138.9, 151.5), double bond (δ_C 124.8 and 135.0), and ester carbonyl (δ_C 170.9) was suggested, showing that this compound should be tetracyclic. Therefore, the ester group was determined to be a 2′,3′-epoxy-2′-methylpropanoyloxy group, supported by the chemical shifts of δ 2.15 and 2.91 (both d, *J*=6.3 Hz). Its stereochemistry was determined by NOESY, as shown in Fig. 5. The configuration at C-2′ was not determined.

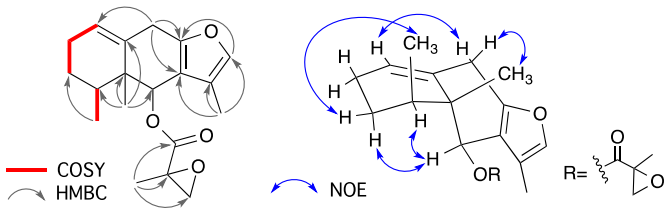

Fig. 5. Selected 2D correlations of compound **4**.

The molecular formula of penultimate compound **5** was determined to be $C_{19}H_{24}O_6$ (by HRMS and ^{13}C NMR) and the IR spectrum indicated the presence of a hydroxy (3500–3700 cm^{-1}), strained lactone (1795 cm^{-1}), ester (1724 cm^{-1}), and double bond (1640 cm^{-1}). ^{13}C NMR showed the presence of three methyl, five methylene (one sp^2), four methine (one sp^2), and seven quaternary carbon (two carbonyl and two alkene) atoms (Table 2). Therefore, this compound should be tetracyclic. The COSY and HMBC correlation shown in Fig. 6 suggested a bakkane skeleton. The acyloxy group substituted at C-6 was determined to be a 2′-hydroxymethylacryloyloxy group. The NOE between H_3-14 and H-9β indicated the conformation as shown in Fig. 6. Because of the presence of an NOE between H_3-13 and H-9β, the carbonyl carbon at C-8 should be in the α-side. At the same time, H_3-13 was determined to be in the α-side and the epoxide oxygen in the β-side (Fig. 6). The configurations of the acyloxy group at C-6 and the methyl group at C-4 were determined to be β, because the NOE between H-4 and H-6 was observed.

Compound **6** exhibited a quasi-molecular ion peak at *m*/*z* 417 $[M+Na]^+$ (FABMS) and its molecular formula was determined to be $C_{21}H_{30}O_7$. The presence of a lactone (1742 cm^{-1}), ester (1718 cm^{-1}), and hydroxy groups (3420–3200 cm^{-1}) was suggested by the IR spectrum. ^1H NMR showed the presence of a triplet methyl, doublet methyl, and two singlet methyl groups, three oxymethine groups, two oxymethylene groups, and two exomethylene groups (Table 1). ^{13}C NMR showed the presence of four methyl, six methylene (two sp^2), four methine, and seven quaternary carbon (two carbonyl and two alkene) atoms (Table 2). These results as well as the COSY and HMBC correlations (Fig. 7) indicated that this compound was an 8,9-seco eremophilane with a 2′-hydroxymethylacryloyloxy group substituted at C-6. C-12 was revealed to be a carbon substituted with two oxygen atoms, one being an ethoxy group. The stereochemistry of the substituents in the six-membered ring was determined by NOESY. The conformation of this part was suggested as shown in Fig. 7 because the NOE was detected between H-6 and H-3α, between H_3-15 and H-2β, and others. The conformation of the ring A part was presumably forced by the presence of a hydrogen bond between OH (C-1) and H-6, although the hydroxy proton was not observed in ^1H NMR. However, the signal of H-6 appeared at δ 6.81 (in $CDCl_3$), a lower field than a normal position for an oxymethine proton,[14] indicating the presence of a hydrogen bond. Secoeremopetasitolide A (**7**) was reported to have a conformation **7a** shown in Fig. 8 and C-6 was substituted in the axial position,

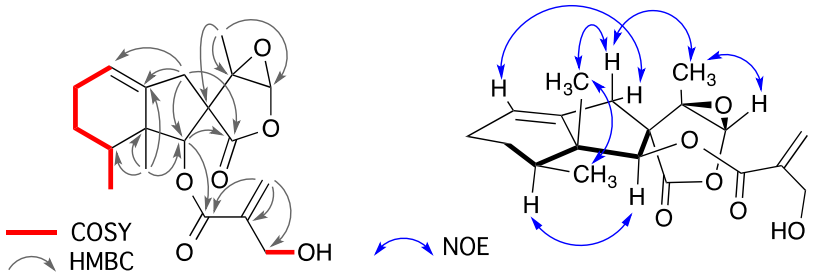

Fig. 6. Selected 2D correlations of compound **5**.

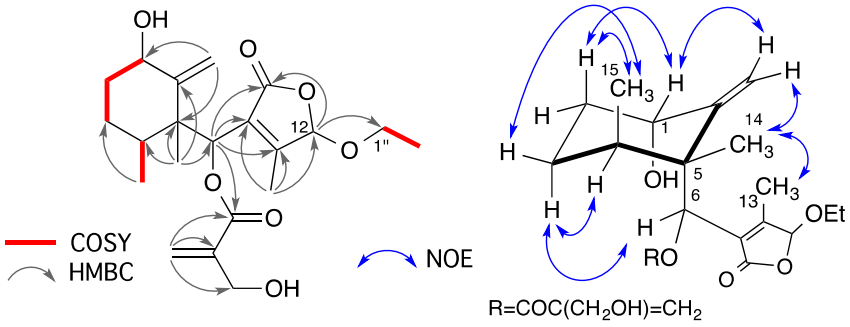

Fig. 7. Selected 2D correlations of compound **6**.

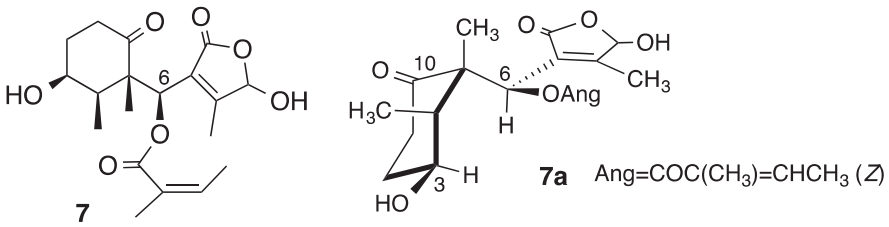

Fig. 8. The structure and conformation of secoeremopetasitolide A (**7**).

which was presumably due to the 1,3-diaxial repulsion present in the flipped conformation.[14] The signal of H-6 of **7** was observed at δ 6.23, a normal position for an oxymethine proton. The configuration at C-6 was deduced to be *S** according to the presence of the NOEs between H-6 and H-3α and between H_3-14 and H_3-13. These results were similar to those of secovirgaurenol.[2b] However, the configuration at C-12 was not determined. Compound **6** was established to be secovirgaurenol 6-*O*-(2′-hydroxymethyl) acrylate.[2b]

The other compounds isolated were identified to be 6β-(2′-hydroxymethylacryloyloxy)furanoeremophil-1(10)-ene (**8**)[9] and 4-methoxy-6-methyl-2-geranylphenol (**9**),[15] by comparing the spectroscopic data with those reported. All but compound **3** was derivative of 6-hydroxyeuryopsin and all had a 2′-hydroxymethylacryloyl group, except for compound **4**. However, the acyl group of compound **4** was also related to a 2′-hydroxymethylacryloyl group.

We previously reported that *L. dictyoneura* was highly diverse.[9] Approximately half of the collected samples were the furanoeremophilane-producing type, and of these, a sample collected near Deqin city, Yunnan, produced 6-(2′-hydroxymethylacryloyloxy)furanoeremophil-1(10)-ene and -epoxide derivatives (type 6), which are very close to compounds **1**–**4**. Compound **8** was a common component to *L. brassicoides* and type 6 of *L. dictyoneura*.[9] However, since the locations of the present samples were far from Deqin city, it was unlikely that type 6 of *L. dictyoneura* and the present *L. brassicoides* samples were related.

The products isolated here were also similar to those of type H of *L. virgaurea*, from which 6-hydroxyeuryopsin (furanoeremophil-1(10)-en-6-ol) and its derivatives were isolated.[2b,11] In addition, compound **6** was a 6-acylated derivative of secovirgaurenol, a component of type H of *L. virgaurea*.[2b] 6-Oxygenated furanoeremophil-1(10)-ene derivatives were also previously isolated from *Ligularia pleurocaulis* (Franch.) Hand.-Mazz.[8] and *Ligularia cyathiceps* Hand.-Mazz.[6] The production of these compounds represents one of the major lineages among furanoeremophilane-producing species.

2.1. Biological activities

Cytotoxic activities of these compounds against HeLa and HL-60 cells were assayed by MTT method according to the standard protocol. Compound **5** showed moderate cytotoxicities, IC_{50} 35.0 and 6.7 μM, against HeLa and HL-60, respectively. Compounds **1**–**4**, **6**, **8**, and **9** exhibited weak activities, IC_{50} 15–29 μM, against HL-60. Against HeLa, activities were weak for compounds **1**–**3**, IC_{50} 37–49 μM, and no activity for compounds **4**, **6**, **8**, and **9**, IC_{50}>60 μM.

3. Conclusion

We investigated the chemical constituents of two samples of *L. brassicoides* and found eight compounds, six of which were new. The terpenoid constituents had a 2-hydroxymethylacryloyl group and its congener. The compounds isolated were similar to those of type H of *L. virgaurea*, but differed slightly because the acyl group at C-6 had an oxymethylene group. Compound **5** exhibited moderate activities against HL-60 and HeLa cells.

4. Experimental

4.1. General

Specific rotations and CD spectra were measured on a JASCO P-1030 and JASCO J-725 auto-recording polarimeter; IR spectra, on a SHIMADZU FT/IR-8400S spectrophotometer (samples were absorbed on a powdered KBr surface and measured with the diffusion reflection method); ^{1}H and ^{13}C NMR spectra, on a Varian 400 MR (400 MHz and 100 MHz, respectively) spectrometer. Mass spectra, including high-resolution, were recorded on a JEOL JMS-700 MStation instrument. Chemcopak Nucleosil 50-5 (4.6×250 mm) with a hexane/EtOAc solvent system was used for HPLC (JASCO pump system). Silica gel BW-127ZH (100–270 mesh, Fuji Silysia) was used for column chromatography. Silica gel 60 F_{254} plates (Merck) were used for TLC.

4.2. Plant material

Two samples of *L. brassicoides* Hand.-Mass., were collected in Muli and Jiulong Counties, Sichuan (3700 m for both samples) in August 2007. The species were identified by G.X., one of the authors, and the voucher specimens, No. 2007-017 and 2007-40, respectively, were deposited in the Herbarium of the Kunming Institute of Botany.

4.3. Extraction and isolation

Sample 1 (dry weight, 2.9 g, voucher No. 2007-40) was cut into pieces and extracted with EtOAc at rt. Extracts (295.0 mg) were obtained after evaporation of the solvent. The residue was subjected to silica gel column chromatography (EtOAc/hexane in gradient) to afford five fractions. Each fraction was further purified by HPLC (Nucleosil 50-5, EtOAc/hexane, and TSK gel G_{1000H}HR, EtOAc) to give **1** (2.5 mg), **2** (1.2 mg), **3** (4.6 mg), **4** (2.1 mg), **5** (5.0 mg), **6** (1.7 mg), **7** (1.6 mg), and **8** (4.2 mg).

Sample 2 (dry weight, 1.4 g, voucher No. 2007-17) was similarly extracted with EtOAc (extracts 37.0 mg) and the residue was purified by silica gel column chromatography (EtOAc/hexane in gradient) and HPLC (Nucleosil 50-5, 40% EtOAc/hexane) to give **5** (0.5 mg).

4.4. Spectroscopic data of the new compounds

4.4.1. Compound ***1****.* $[\alpha]_D^{23}$ −5.9 (*c* 0.25, EtOH). FTIR 3600–3400, 1807, 1718, 1637 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. MS (CI) *m*/*z* 349 $[M+H]^+$, 331, 265, 247 (base), 219, 191, 87. HRMS (CI) obsd *m*/*z* 349.1647 $[M+H]^+$. Calcd for $C_{19}H_{25}O_6$ 349.1651.

4.4.2. Compound ***2****.* $[\alpha]_D^{22}$ −7.4 (*c* 0.19, EtOH). FTIR 3600–3400, 1718, 1670 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. MS (CI) *m*/*z* 331 $[M+H]^+$ 228 (base), 41. HRMS (CI) obsd *m*/*z* 331.1544 $[M+H]^+$. Calcd for $C_{19}H_{23}O_5$ 331.1546.

4.4.3. Compound ***3****.* $[\alpha]_D^{22}$ −26.4 (*c* 0.46, EtOH). FTIR 3500–3300, 1712, 1693, 1666 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. MS (CI) *m*/*z* 333 $[M+H]^+$ (base), 248, 231. HRMS (CI) obsd *m*/*z* 333.1693 $[M+H]^+$. Calcd for $C_{19}H_{25}O_5$ 333.1702. CD [θ] (nm) (EtOH): −6200 (202), +18,000 (215), −13,600 (233), −4200 (271), +2500 (310).

4.4.4. Compound ***4****.* $[\alpha]_D^{23}$ −7.0 (*c* 0.21, EtOH). FTIR 1733, 1169, 1140, 1092, 953, 941 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. MS (CI) *m*/*z* 317 $[M+H]^+$, 215 (base), 172. HRMS (CI) obsd *m*/*z* 317.1752 $[M+H]^+$. Calcd for $C_{19}H_{25}O_4$ 317.1753.

4.4.5. Compound ***5****.* $[\alpha]_D^{22.0}$ +52.1 (*c* 0.25, EtOH). FTIR 3500–3370, 1795, 1724, 1640 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. MS (CI) *m*/*z* 349 $[M+H]^+$, 247 (base), 219, 203, 191. HRMS (CI) obsd *m*/*z* 349.1651 $[M+H]^+$. Calcd for $C_{19}H_{25}O_6$ 349.1651.

4.4.6. Compound ***6****.* $[\alpha]_D^{22.2}$ +27.5 (*c* 0.17, EtOH). FTIR 3420–3200, 1742, 1718 cm^{-1}. ^{1}H and ^{13}C NMR; see Tables 1 and 2. MS (FAB) *m*/*z* 417 $[M+Na]^+$ 307, 154 (base), 136. HRMS (CI) obsd *m*/*z* 395.2040 $[M+H]^+$. Calcd for $C_{21}H_{31}O_7$ 395.2070. CD [θ] (nm) (EtOH): −4800 (206), +20,400 (218), +12,300 (243).

4.5. Biological activities

See Ref. 16.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.10.003. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Liu, J. Q.; Wang, Y. J.; Wang, A. L.; Ohba, H.; Abbott, R. *Mol. Phylogenet. Evol.* **2006**, *38*, 31–49.
2. (a) Saito, Y.; Taniguchi, M.; Komiyama, T.; Ohsaki, A.; Okamoto, Y.; Gong, X.; Kuroda, C.; Tori, M. *Tetrahedron* **2013**, *69*, 8505–8510; (b) Saito, Y.; Takashima, Y.; Kamada, A.; Suzuki, Y.; Suenaga, M.; Okamoto, Y.; Matsunaga, Y.; Hanai, R.; Kawahara, T.; Gong, X.; Tori, M.; Kuroda, C. *Tetrahedron* **2012**, *68*, 10011–10029; (c) Tori, M.; Honda, K.; Nakamizo, H.; Okamoto, Y.; Sakaoku, M.; Takaoka, S.; Gong, X.; Shen, Y.; Kuroda, C.; Hanai, R. *Tetrahedron* **2006**, *62*, 4988–4995.
3. (a) Shimizu, A.; Suzuki, Y.; Torihata, A.; Hanai, R.; Saito, Y.; Tori, M.; Gong, X.; Kuroda, C. *Nat. Prod. Commun.* **2012**, *7*, 431–434; (b) Tori, M.; Watanabe, A.; Matsuo, S.; Okamoto, Y.; Tachikawa, K.; Takaoka, S.; Gong, X.; Kuroda, C.; Hanai, R. *Tetrahedron* **2008**, *64*, 4486–4495.
4. Tori, M.; Nakamizo, H.; Mihara, K.; Sato, M.; Okamoto, Y.; Nakashima, K.; Tanaka, M.; Saito, Y.; Sono, M.; Gong, X.; Shen, Y.; Hanai, R.; Kuroda, C. *Phytochemistry* **2008**, *69*, 1158–1165.
5. (a) Saito, Y.; Hattori, M.; Iwamoto, Y.; Takashima, Y.; Mihara, K.; Sasaki, Y.; Fujiwara, M.; Sakaoku, M.; Shimizu, A.; Chao, X.; Kuroda, C.; Gong, X.; Hanai, R.; Tori, M. *Tetrahedron* **2011**, *67*, 2220–2231; (b) Tori, M.; Okamoto, Y.; Tachikawa, K.; Mihara, K.; Watanabe, A.; Sakaoku, M.; Takaoka, S.; Tanaka, M.; Gong, X.; Kuroda, C.; Hattori, M.; Hanai, R. *Tetrahedron* **2008**, *64*, 9136–9142.
6. Nagano, H.; Torihata, A.; Matsushima, M.; Hanai, R.; Saito, Y.; Baba, M.; Tanio, Y.; Okamoto, Y.; Takashima, Y.; Ichihara, M.; Gong, X.; Kuroda, C.; Tori, M. *Helv. Chim. Acta* **2009**, *92*, 2071–2081.
7. Hanai, R.; Gong, X.; Tori, M.; Kondo, S.; Otose, K.; Okamoto, Y.; Nishihama, T.; Murota, A.; Shen, Y.; Wu, S.; Kuroda, C. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1302–1308.
8. Nagano, H.; Iwazaki, Y.; Gong, X.; Shen, Y.; Kuroda, C.; Hanai, R. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 300–304.
9. Nagano, H.; Iwazaki, Y.; Matsushima, M.; Sato, M.; Gong, X.; Shen, Y.; Hirota, H.; Kuroda, C.; Hanai, R. *Chem. Biodivers.* **2007**, *4*, 2874–2888.
10. Tori, M.; Tanio, Y.; Okamoto, Y.; Saito, Y.; Gong, X.; Kuroda, C.; Hanai, R. *Heterocycles* **2008**, *75*, 2029–2034.
11. Kuroda, C.; Hanai, R.; Nagano, H.; Tori, M.; Gong, X. *Nat. Prod. Commun.* **2012**, *7*, 539–548.
12. Liu, S.; Illarionova, I. D. Ligularia In*Wu, Z. Y., Raven, P. H., Hong, D. Y., Eds.. Flora of China*; Science and Missouri Botanical Garden: Beijing and St. Louis, MO, 2011; Vols. 20–21, pp 376–415.
13. Saito, Y.; Ichihara, M.; Takiguchi, K.; Tanio, Y.; Okamoto, Y.; Hanai, R.; Kuroda, C.; Kawahara, T.; Gong, X.; Tori, M. *Phytochemistry* **2013**, *96*, 184–190.
14. Yaoita, Y.; Kikuchi, M. *Phytochemistry* **1996**, *42*, 751–755 The ^{1}H NMR spectrum of compound **6** was measured in $CDCl_3$ to compare the chemical shifts with those of secoeremopetasitolide A (**7**).
15. Nagano, H.; Hanai, R.; Yamada, H.; Matsushima, M.; Miura, Y.; Hoya, T.; Ozawa, M.; Fujiwara, M.; Kodama, H.; Torihata, A.; Onuki, H.; Nezu, Y.; Kawai, S.; Yamazaki, Y.; Hirota, H.; Saito, Y.; Tori, M.; Ohsaki, A.; Gong, X.; Kuroda, C. *Chem. Biodivers.* **2012**, *9*, 789–805.
16. Saito, Y.; Sasaki, Y.; Komiyama, T.; Ohsaki, Y.; Okamoto, Y.; Gong, X.; Kuroda, C.; Tori, M. *Tetrahedron* **2014**, *70*, 5878–5883.