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Five new eremophilane derivatives from *Ligularia sagitta*

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Abstract—Ligulasagitins A–E (**1**–**5**), five new eremophilane-derived metabolites possibly formed via a Diels–Alder reaction in the biosynthetic process, were isolated from *Ligularia sagitta* Maxim. Among them **1** and **2** possess a novel C19 skeleton, **4** and **5** are two novel dimeric eremophilane type derivatives. Their structures were determined by extensive spectroscopic analysis and the structure of **2** was also confirmed by a single-crystal X-ray diffraction analysis. Furthermore, **4** and **5** showed weak cytotoxic activity against HL-60 (human promyelocytic leukemia) cells.

1. Introduction

The genus *Ligularia* has been taxonomically placed in the Compositae with ca. 100 species distributed within China, more than 20 species have long been used for folk medicines. Their roots, stems, leaves, and flowers possess efficacies of antipyretic, relieving phlegm and cough, invigorating circulation of blood, and soothing pain.[1] The main components of them are eremophilane sesquiterpenes and pyrrolizidine alkaloids with strong physiological actvities.[2] In our long-standing interest in the study of biodiversity and searching for bioactive compounds from several *Ligularia* species,[3] those results showed that the components from the same genus, even from the same species, displayed a remarkable differences because of the differences of ecological environments and collection seasons.[4] In the present study, five new eremophilane derivatives Ligulasagitins A–E (**1**–**5**) were isolated from the roots of *Ligularia sagitta* collected from Gannan Tibet Autonomous Region (S. A. 2000–3800 m), Gansu province of PR China. Herein we report isolation, structure elucidation, biogenetic transformation, and cytotoxicity evaluation of compounds **1**–**5** (Fig. 1).

2. Results and discussion

Ligulasagitin A (**1**) was obtained as a white amorphous powder. Its HRESIMS gave an ion peak of $[M+H]^+$ at *m/z* 349.1644 consistent with the molecular formula of $C_{19}H_{24}O_6$ (calcd 349.1646), which accounted for eight degrees of unsaturation. The IR absorptions at 1629, 1664, and 1699 cm^{-1} showed the presence of an α,β,α′,β′-unsaturated ketone moiety, which was further supported by ^1H NMR spectral signals at δ 6.18 (s, H-9), 7.24 (s, H-6), and ^{13}C NMR (DEPT) spectral signals at δ 156.1 (C-6), 135.0 (C-7), 126.9 (C-9), 165.9 (C-10), 186.2 (C-8). The remaining three degrees of unsaturation except for another trisubstituted double bond at δ 7.69 (s, H-18), 154.2 (C-18), 103.9 (C-17) and a carboxyl group at δ 171.6 (C-19) suggested

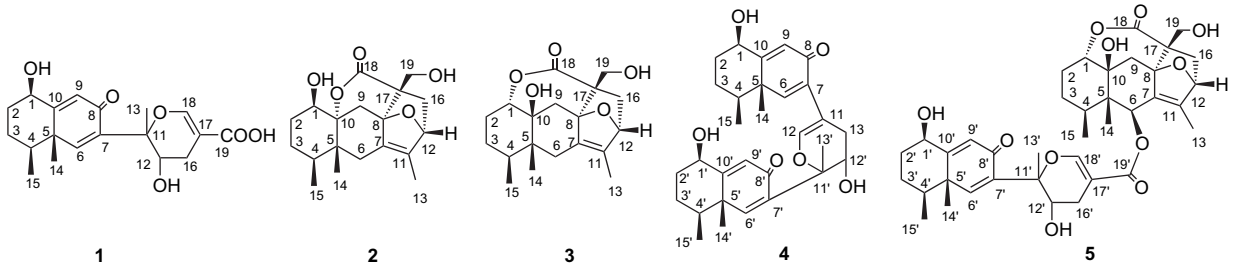

Figure 1. Structures of compounds **1**–**5**.

Keywords: *Ligularia sagitta*; Novel carbon skeleton; X-ray diffraction; Cytotoxic activity.
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a tricyclic structure for **1**. The ^{1}H NMR spectrum of **1** showed three methyl group signals at δ 1.56 (s, H_3-13), 1.38 (s, H_3-14), and 1.11 (d, J=6.3 Hz, H_3-15), suggesting that **1** was an eremophilane derivative.[4]

The unusual 19-carbon skeleton, as in compound **1**, could be identified as two partial sequences by comprehensive analysis of 2D NMR data, including the results of COSY, HSQC, and HMBC experiments (Fig. 2): the first part, a carbon skeleton of a series of normal eremophilane sesquiterpenes isolated from *L. sagitta* previously,[4] was revealed by the proton sequence H-1/H_2-2/H_2-3/H-4 deduced from ^{1}H–^{1}H COSY spectrum and the HMBC correlations of H_3-13 with C-7, C-11, C-12, H_3-14 with C-4, C-5, C-6, C-10, H_3-15 with C-3, C-4, C-5, H-6 with C-8, C-11, and H-9 with C-1, C-7; the another part could be identified as a methylacrylic acid by the HMBC correlations of H-18 with C-17, C-19, C-16, which was also supported by the downfield signals at δ 7.69 (s, H-18), δ 154.2 (C-18), and upfield signal at δ 103.9 (C-17), as a result of the oxygenation of CH-18 and p–π conjugation. Most importantly, the HMBC correlations of H-18 with C-11, H_2-16 with C-11, C-12, and proton sequence H-12/H_2-16 revealed by ^{1}H–^{1}H COSY spectrum, suggested that the two partial structures were connected by forming a 2H-pyran ring which was also confirmed by the IR absorptions at 1263, 1215 cm^{-1} and 1055, 1022 cm^{-1}, and this kind of carbon skeleton was unprecedented.

Stereochemically, in the biogenetic consideration of eremophilane derivatives isolated from Compositae species, the methyls at C-4 and C-5 were both assigned the β-orientation.[5,6a] The broad single signal at δ 4.56 (br s, H-1) showed H-1 to be equatorial α-orientation, because the alternative of axial β-oriented H-1 must had one large coupling pattern with the coupling constant of at least 7 Hz, and NOESY correlation of H-12/Me-13 indicated that Me-13 and OH-12 were in the different side of the 2H-pyran plane.

Ligulasagitin B (**2**), colorless needles, its molecular formula was deduced as $C_{19}H_{26}O_5$ from HRESIMS at *m*/*z* 691.3456 $[2M+Na]^+$. The ^{13}C NMR and DEPT spectra of **2** displayed the signals of a tetrasubstituted double bond at δ 132.9 (C-7), 143.9 (C-11), and a carboxyl group at δ 172.9 (C-18), thus, the left five of seven degrees of unsaturation suggested **2** to be a compound with five rings. The ^{1}H NMR of **2** showed the methyl signals for an eremophilane skeleton at δ 1.71 (br s, H_3-13), 1.18 (s, H_3-14), and 0.84 (d, J=6.3 Hz, H_3-15), and also indicated that C-8 and C-17 must be wholly substituted to account for two pairs of doublets for H_2-9 at δ 2.48 (d, J=12.9 Hz, H-9a), 2.93 (d, J=12.9 Hz, H-9b) and H_2-19 at δ 3.91 (d, J=11.1 Hz, H-19a), 3.85 (d, J=10.5 Hz, H-19b).

^{1}H–^{1}H COSY spectrum showed two spin coupling systems a (C-1, C-2, C-3, and C-4) and b (C-12 and C-16) as drawn with bold bonds (Fig. 3). Careful analysis of 2D NMR

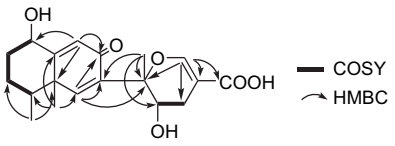


Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations of **1**.

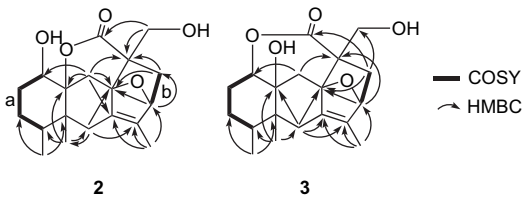


Figure 3. ^{1}H–^{1}H COSY and key HMBC correlations of **2** and **3**.

spectra revealed that **2** also possessed a carbon skeleton of the normal eremophilane sesquiterpene, which could be supported by HMBC correlations of H_3-13 with C-7, C-11, C-12, H_3-14 with C-4, C-5, C-6, C-10, H_3-15 with C-3, C-4, C-5, H_2-6 with C-5, C-7, C-8, C-10, C-11, and H_2-9 with C-1, C-7, C-8, C-10. The HMBC correlations of H_2-16 with C-8, C-11, C-17, C-18, C-19, H_2-9 with C-17, and H_2-19 with C-8, C-16, C-18 suggested that an additional carbon chain with a carboxyl connected to C-8 and C-12, and the presence of an oxygen bridge between C-8 and C-12 could be revealed by HMBC correlations of H-12 with C-7, C-8, and C-17. Furthermore, the downfield shift of C-10 at δ 91.5 indicated that the ester moiety is connected to C-10 by comparison with the ^{13}C NMR data of the known compounds.[4d]

The relative chemistry of compound **2** was determined by the combination of biogenetic considerations of eremophilane derivatives and the coupling pattern of H-1. The Me-14 and Me-15 were biogenetically β; and a broad single signal at δ 4.00 (br s, H-1) in ^{1}H NMR spectrum of **2** revealed, with a help from a model of the molecule, that H-1 must have the equatorial α-orientation. However, the relative stereochemistry of the olide ring cannot be fully determined by this way.

A single X-ray diffraction analysis (Fig. 4) was then carried out in order to determine the structure of **2**. The X-ray structure of **2** demonstrated the linkage and α-orientation of the olide ring, and it also showed that the CH_2-19 and H-12 are both β-oriented. Based on the above findings, the structure, including the relative stereochemistry of **2**, was

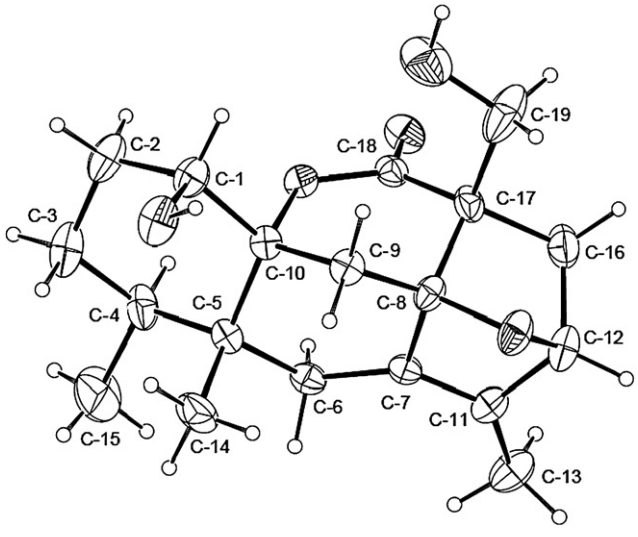


Figure 4. The X-ray structure of compound **2**.

unambiguously elucidated as a novel eremophilane derivative with 19-carbon skeleton.

Ligulasagitin C (**3**), white amorphous powder, had the same molecular formula with that of **2** as deduced from HRESIMS at *m/z* 352.2122 $[M+NH_4]^+$. The ^1H NMR spectrum showed signals for three methyls at δ 1.68 (br s, H_3-13), 1.07 (s, H_3-14), 1.20 (d, J=7.2 Hz, H_3-15), an oxygenated methylene at δ 4.07 (d, J=10.0 Hz, H-19a), 3.99 (d, J=10.0 Hz, H-19b), and two oxygenated methines at δ 4.55 (d, J= 3.6 Hz, H-12), 4.79 (br s, H-1), and the ^{13}C NMR (DEPT) spectra of **3** displayed 19-carbon signals for the skeleton of **3**. The ^1H and ^{13}C NMR of compound **3** were similar to those of **2** except for that the C-1 of **3** shifted downfield to δ 84.2 from δ 74.2 while the C-10 shifted upfield to δ 74.4 from δ 91.5, which revealed that an ester moiety was connected to C-1 in **3** instead of C-10 in **2**. Careful analysis of the spectroscopic data of COSY, HSQC, and HMBC experiments (Fig. 3) enabled the complete planar structure of **3** to be assigned. Stereochemically, with a help from a model of the molecule, the broad single signal of H-1 at δ 4.80 (br s), with possible small coupling pattern, showed H-1 to be β-orientation, and the ester moiety (C-18, C-17, and C-16) must be α-oriented because of an impossibly large torsion in formation of the alternative of βC-17/βC-16. Furthermore, the structure of compound **3** was confirmed by the presence of the same series of compounds Ligulaverins A–E which have been isolated form *Ligularia veitchiana*, with the stereochemistry of them confirmed by X-ray crystallographic study.[6] Thus, the structure of compound **3** was established.

Ligulasagitin D (**4**), optically active oil, showed the molecular formula of $C_{30}H_{38}O_6$ as determined by HRESIMS at *m/z* 495.2733 $[M+H]^+$. The IR spectrum exhibited absorption bands at 1622, 1658, and 1709 cm^{-1}, along with the ^1H and ^{13}C NMR (DEPT) spectra displayed at δ 6.73 (s, H-6), 6.13 (s, H-9), 7.28 (s, H-6′), 6.18 (s, H-9′), and δ 150.7 (C-6), 133.4 (C-7), 127.0 (C-9), 164.5 (C-10), 186.5 (C-8), 156.7 (C-6′), 135.5 (C-7′), 127.0 (C-9′), 166.2 (C-10′), 187.1 (C-8′), indicating the presence of two α,β,α′,β′-unsaturated ketone moieties. Most interestingly, the ^1H and ^{13}C NMR spectra of **4** mainly displayed signals in pairs, indicating that compound **4** might be a dimeric derivative. Furthermore, the ^1H and ^{13}C NMR spectra showed the presence of a trisubstituted double bond at δ 7.43 (s, H-12) and δ 142.3 (C-12), 107.4 (C-11), an oxygenated methine at δ 4.29 (dd, J=6.6, 4.5 Hz, H-12′), and an oxygenated quaternary carbon at δ 79.8 (C-11′), all these evidences revealed the presence of a 2H-pyran ring by comparison with the 1D NMR data of compound **1**.

The key HMBC correlations and the proton sequences revealed by ^1H–^1H COSY spectrum showed two normal eremophilane skeletons with an α,β,α′,β′-unsaturated ketone moiety (Fig. 5). The HMBC correlations of H-12 with C-7, C-11′, C-13, H_3-13′ with C-7′, C-12′, and H-6′ with C-11′, C-8′, together with the proton sequence of H-12′/H-13′ revealed by ^1H–^1H COSY spectrum, confirmed that the two eremophilane sesquiterpenes were connected by forming a 2H-pyran ring, similar to the partial structure of compound **1**.

The relative configuration of both H-1 at δ 4.52 (dd, J=3.0, 3.0 Hz) and H-1′ at δ 4.55 (dd, J=3.0, 3.0 Hz) was determined to be β-orientation by their coupling pattern. Furthermore, the ^1H and ^{13}C NMR data and the coupling pattern of the 2H-pyran moiety of **4** were similar to those of compound **1**, indicating that Me-13′ and OH-12′ were also in the different side of the 2H-pyran plane.

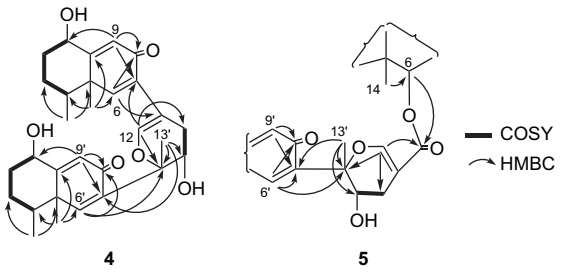
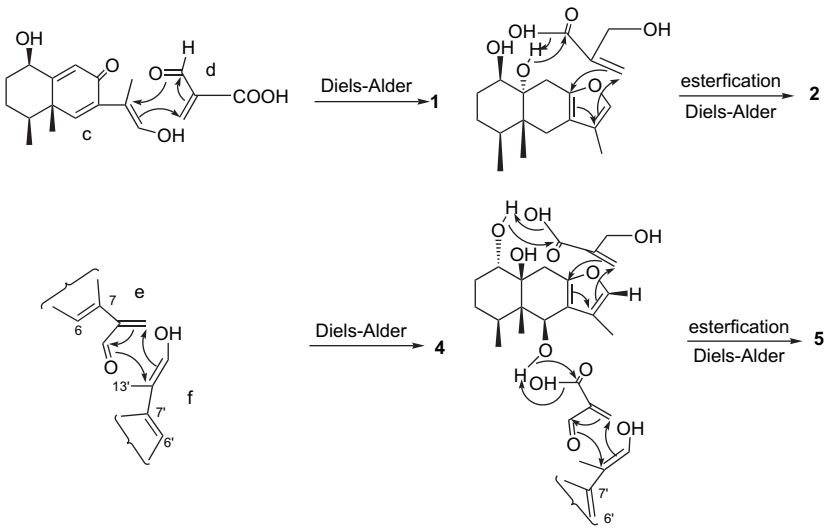


Figure 5. ^1H–^1H COSY and key HMBC correlations of **4** and **5**.

The structure determination of Ligulasagitin E (**5**) was established by careful analysis of its 1D and 2D NMR spectroscopic data and comparison of the spectral data of **5** with those of **1** and **3**. The molecular formula was determined as $C_{38}H_{48}O_{11}$ by HRESIMS at *m/z* 698.3519 $[M+NH_4]^+$. The signals in ^1H NMR spectrum of **5** at δ 1.34 (s, H_3-14), 1.45 (d, J=7.2 Hz, H_3-15), 1.60 (d, J=2.0 Hz, H_3-13), 3.92 (d, J=11.2 Hz, H-19a), 3.83 (d, J=11.2 Hz, H-19b), 4.44 (d, J=4.4 Hz, H-12), 5.10 (br s, H-1) showed that the structure of **5** was similar to **3**, and the similarity to **1** also could be revealed at δ 1.09 (d, J=6.8 Hz, H_3-15′), 1.37 (s, H_3-14′), 1.46 (s, H_3-13′), 6.05 (s, H-9′), 7.29 (s, H-6′), and 7.65 (s, H-18′). The HMBC correlations of H-6′ with C-8′, C-11′, H_3-13′ with C-7′, C-12′, and H-18′ with C-11′, C-16′, together with the proton sequence H-12′/H_2-16′ revealed by ^1H–^1H COSY spectrum (Fig. 5), also indicated the presence of a 2H-pyran moiety similar to that in **1**, and the HMBC correlations of H_2-6, H_2-16′, H-18′ with C-19′ further suggested that compound **5** was a dimeric eremophilane type derivative by esterification of **1** and a C-6 oxygenated derivative of **3**. Stereochemically, H-6 must have the α-orientation to allow the homoallylic coupling with Me-13 at δ 1.60 (d, J= 2.0 Hz).[6a] The similarities of 1D NMR data of 2H-pyran moiety of compound **5** to that of compound **1** also showed the same stereochemical structure of the 2H-pyran moiety with that in **1**.

Compounds **1**–**5** were tested for their cytotoxic activity against HL-60 (human promyelocytic leukemia), SMMC-7721 (human hepatoma), and HeLa (human cervical carcinoma) cells according to the sulforhodamine B (SRB) method[7] (use vincristine sulfate as a positive control with IC_{50} values 11.2 μg/mL against HL-60, 26.7 μg/mL against SMMC-7721, and 8.3 μg/mL against HeLa) as reported previously. Compounds **1**–**3** are not bioactive (IC_{50}> 100 μg/mL) against the three human tumor cells, compounds **4** and **5** showed weak cytotoxicity against HL-60 cells with IC_{50} values of 77.6 and 60.0 μg/mL, respectively, and showed no bioactive (IC_{50}>100 μg/mL) against SMMC-7721 and HeLa cells.

In addition, a possible biosynthetic pathway for compounds **1**, **2**, **4**, and **5** are shown in Scheme 1, which illustrates that all these five new compounds (**1**–**5**) could be identified as a series of sesquiterpenoid-derived metabolites possibly formed



Scheme 1. Biogenetic pathway proposed for compounds **1**, **2**, **4**, and **5**.

via a Diels–Alder reaction. Compound **1** mainly showed a common 15-carbon skeleton of eremophilane sesquiterpene except for an acrylic acid moiety, thus, the most reasonable route to this kind of unusual compound was via c, of which the acetated derivative has previously been isolated from *L. sagitta*,[4a] and the existence of d (2-formylacrylic acid), followed by a Diels–Alder reaction, would then yield compound **1**. Compound **4** was a dimeric derivative formed by two eremophilane type derivatives e and f, followed by a Diels–Alder reaction similar to the route of compound **1**. Compounds **2** and **3** also followed a Diels–Alder reaction when the double bond of 2-formylacrylic acid connected to C-8/C-12, and followed an esterification to form the lactone, as reported previously.[6] Furthermore, formation of compound **1**, followed by an esterification with the C-6 oxygenated derivative of compound **3**, would then yield compound **5**. The proposed Diels–Alder reaction further supports the deduction that enzyme-catalyzed Diels–Alder reaction does occur in biosynthetic pathways.[8]

3. Experimental

3.1. General experimental procedures

Melting points were determined on an X-4 digital display micromelting point apparatus, and were uncorrected. Optical rotations were measured on a Perkin Elmer 341 polarimeter. IR spectra were taken on a Nicolet NEXUS 670 FT-IR spectrometer TU-1901. UV spectra were taken on a Shimadzu spectrometer UV-240. NMR spectra were recorded on a Varian Mercury plus-300 and a Varian Mercury plus-400 NMR spectrometers with TMS as an internal standard. HRESIMS data were measured on a Bruker Daltonics

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**3**

	1[a]		**2**[a]		**3**[b]	
	δ ^{13}C[c]	δ ^{1}H[d] m, Hz	δ ^{13}C[c]	δ ^{1}H[d] m, Hz	δ ^{13}C[c]	δ ^{1}H[d] m, Hz
1	73.6 d	α 4.56 br s	74.2 d	α 4.00 br s	84.2 d	β 4.79 br s
2	34.7 t	a 2.04 m, b 1.63 m	30.1 t	a 2.20 m, b 1.65–1.70 m	23.9 t	a 2.35 m, b 1.68 m
3	25.2 t	a 1.92 m, b 1.47 m	24.9 t	a 1.50–1.60 m, b 1.30–1.40 m	23.2 t	a 2.31 m, b 1.30 m
4	42.3 d	α 1.47 m	36.8 d	α 1.90 m	38.4 d	α 1.54 m
5	44.4 s		41.4 s		38.6 s	
6	156.1 d	7.24 s	35.7 t	a 1.70–1.80 m, b 2.34 br d, 18.0	34.3 t	a 1.95 d, 13.6, b 2.58 br d, 13.6
7	135.0 s		132.9 s		135.1 s	
8	186.2 s		87.0 s		86.3 s	
9	126.9 d	6.18 s	27.7 t	a 2.48 d, 12.9, b 2.93 d, 12.9	36.5 t	a 2.23 d, 13.2, b 2.47 d, 13.2
10	165.9 s		91.5 s		74.4 s	
11	82.3 s		143.9 s		142.8 s	
12	67.3 d	4.34 dd, 5.4, 4.2	83.0 d	β 4.70 d, 3.9	81.7 d	β 4.55 d, 3.6
13	23.4 q	1.56 s	10.1 q	1.71 br s	10.4 q	1.68 br s
14	18.8 q	1.38 s	16.5 q	1.18 s	22.6 q	1.07 s
15	16.4 q	1.11 d, 6.3	15.8 q	0.84 d, 6.3	17.6 q	1.20 d, 7.2
16	26.4 t	a 2.55 dd, 18.0, 4.5, b 2.18 dd, 18.0, 5.1	36.2 t	a 2.05 dd, 12.3, 3.9, b 1.70–1.80 m	40.6 t	a 2.15 d, 12.0, b 2.08 dd, 12.0, 4.0
17	103.9 s		54.2 s		59.2 s	
18	154.2 d	7.69 s	172.9 s		173.8 s	
19	171.6 s		66.8 t	a 3.91 d, 11.1, b 3.85 d, 10.5	65.6 t	a 4.07 d, 10.0, b 3.99 d, 10.0

[a] Data were recorded in $CDCl_3$ on a Varian Mercury plus-300 MHz (75 MHz for ^{13}C) with TMS as an internal standard.
[b] Data were recorded in $CDCl_3$ on a Varian Mercury plus-400 MHz (100 MHz for ^{13}C) with TMS as an internal standard.
[c] By DEPT, HSQC, and HMBC experiments.
[d] By ^{1}H–^{1}H COSY and HSQC experiments.

APEX II 47e spectrometer. Silica gel (200–300 mesh) used for column chromatography and silica gel GF_{254} (10–40 μm) used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, PR China. The reversed phase pre-coated TLC plates RP-18 F_{254}s (size 20×20 cm, Schichtdicke 0.25 mm) were supplied by E. Merck Factory, Germany. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

3.2. Plant material

The roots of *L. sagitta* Maxim were collected from Gannan Tibet Autonomous Region (S. A. 2000–3800 m), Gansu province of PR China in August 2005. It was identified by Prof. Guo-Liang Zhang, School of Life Sciences, Lanzhou University. A voucher specimen (No. 20050920) was deposited in the College of Chemistry and Chemical Engineering, Lanzhou University.

3.3. Extraction and isolation

The air-dried roots of *L. sagitta* (10 kg) were pulverized and extracted with mixed solvent (petroleum ether (bp 60–90 °C)/ether/MeOH 1:1:1) three times (7 days each time) at room temperature. The extract was concentrated under reduced pressure, the residue (400 g) was subjected to a silica gel column chromatography, and eluded with a step gradient of petroleum ether/acetone (20:1, 10:1, 5:1, 3:1, and 1:1). Five fractions were collected according to TLC analysis. Fr. 3 (petroleum ether/acetone 5:1, 50 g) was purified by repeatedly chromatographed over a silica gel column with petroleum ether/acetone (5:1) to afford **1** (10 mg), **2** (11 mg), and **5** (11 mg). Fr. 4 (petroleum ether/acetone 3:1, 40 g) after silica gel column chromatography (200–300 mesh, 400 g) with petroleum ether/acetone (3:1) as eluent gave two mixtures, which were then rechromatographed by RP-18 PTLC (H_2O/CH_3OH 1:3, two times each), respectively, to afford **3** (R_f=0.4–0.5, 8 mg) and **4** (R_f=0.6–0.7, 7 mg).

3.3.1. Ligulasagitin A (1). White amorphous powder (10 mg); mp 202–204 °C; $[\alpha]_D^{20}$ −9 (*c* 0.4, $CHCl_3$); UV ($CHCl_3$) λ_{max} 246 nm; IR (film) ν_{max} 3410, 2927, 2856, 1699, 1663, 1629, 1367, 1215, 1161, 1099, 1055, 1022 cm^{-1}; ^1H and ^{13}C NMR data: see Table 1; HRESIMS *m/z* 349.1644 $[M+H]^+$ (calcd for $C_{19}H_{24}O_6$+H, 349.1646).

Table 2. ^1H and ^{13}C NMR spectral data of compounds **4** and **5**

	4[a]		**5**[b]	
	δ ^{13}C[c]	δ ^1H[d] m, Hz	δ ^{13}C[c]	δ ^1H[d] m, Hz
1	73.7 d	α 4.52 dd, 3.0, 3.0	82.5 d	β 5.10 br s
2	34.7 t	a 2.05–2.10 m, b 1.55–1.65 m	23.6 t	a 1.45–1.55 m, b 1.20–1.25 m
3	25.3 t	a 1.90–1.95 m, b 1.58–1.62 m	23.3 t	a 2.38–2.43 m, b 1.38–1.43 m
4	42.0 d	α 1.35–1.40 m	33.2 d	α 1.38–1.42 m
5	43.8 s		44.9 s	
6	150.7 d	6.73 s	69.5 d	α 6.32 br s
7	133.4 s		134.6 s	
8	186.5 s		85.9 s	
9	127.0 d	6.13 s	34.9 t	2.40–2.50 m
10	164.5 s		74.1 s	
11	107.4 s		145.2 s	
12	142.3 d	7.43 s	81.8 d	β 4.44 d, 4.4
13	29.9 q	2.51 dd, 16.5, 4.5	9.8 q	1.60 d, 2.0
14	16.4 q	1.09 d, 6.9	16.2 q	1.34 s
15	19.3 q	1.30 s	16.8 q	1.45 d, 7.2
16	___	___	41.2 t	a 1.92–1.98 m, b 1.75 dd, 12.4, 4.4
17	___	___	60.3 s	
18	___	___	174.1 s	
19	___	___	66.6 t	a 3.92 d, 11.2, b 3.83 d, 11.2
1′	73.6 d	α 4.55 dd, 3.0, 3.0	72.7 d	α 4.48 br s
2′	34.7 t	a 2.05–2.10 m, b 1.55–1.65 m	34.9 t	A 1.98–2.04 m, b 1.48–1.53 m
3′	25.3 t	A 1.90–1.95 m, b 1.58–1.62 m	25.3 t	A 1.92–1.98 m, b 1.38–1.42 m
4′	42.5 d	α 1.35–1.40 m	41.9 d	α 1.35–1.38 m
5′	44.5 s		43.9 s	
6′	156.7 d	7.28 s	154.5 d	7.29 s
7′	135.5 s		135.5 s	
8′	187.1 s		185.7 s	
9′	127.0 d	6.18 s	126.1 d	6.05 s
10′	166.2 s		166.5 s	
11′	79.8 s		81.6 s	
12′	68.7 d	4.29 dd, 6.6, 4.5	65.3 d	4.42 m
13′	29.9 q	1.58 s	22.5 q	1.46 s
14′	16.5 q	1.06 d, 6.6	18.4 q	1.37 s
15′	18.7 q	1.33 s	15.9 q	1.09 d, 6.8
16′	___	___	26.6 t	a 2.53–2.55 m, b 2.27 dd, 17.2, 3.2
17′	___	___	103.6 s	
18′	___	___	153.0 d	7.65 s
19′	___	___	167.2 s	

[a] Data were recorded in $CDCl_3$ on a Varian Mercury plus-300 MHz (75 MHz for ^{13}C) with TMS as an internal standard.
[b] Data were recorded in acetone on a Varian Mercury plus-400 MHz (100 MHz for ^{13}C) with TMS as an internal standard.
[c] By DEPT, HSQC, and HMBC experiments.
[d] By ^1H–^1H COSY and HSQC experiments.

3.3.2. Ligulasagitin B (2). Colorless needles (11 mg); mp 196–198 °C; $[\alpha]_D^{20}$ +4 (*c* 0.2, $CHCl_3$); IR (film) ν_{max} 3455, 3399, 2954, 2925, 1705, 1060, 1031, 936 cm^{-1}; ^1H and ^{13}C NMR data: see Table 1; HRESIMS *m/z* 691.3456 $[2M+Na]^+$ (calcd for $2C_{19}H_{26}O_5$+Na, 691.3453).

3.3.3. Ligulasagitin C (3). White amorphous powder (8 mg); mp 184–186 °C; $[\alpha]_D^{20}$ +3 (*c* 0.3, $CHCl_3$); IR (film) ν_{max} 3445, 3369, 2918, 2857, 1682, 1339, 1178, 1085, 1041, 976 cm^{-1}; ^1H and ^{13}C NMR data: see Table 1; HRESIMS *m/z* 352.2122 $[M+NH_4]^+$ (calcd for $C_{19}H_{26}O_5$+NH_4, 352.2118).

3.3.4. Ligulasagitin D (4). Optically active oil (7 mg); $[\alpha]_D^{20}$ −20 (*c* 0.2, $CHCl_3$); UV ($CHCl_3$) λ_{max} 248 nm; IR (film) ν_{max} 3410, 2927, 2877, 1709, 1658, 1622, 1211, 1157, 1099, 1053, 1019 cm^{-1}; ^1H and ^{13}C NMR data: see Table 2; HRESIMS *m/z* 495.2733 $[M+H]^+$ (calcd for $C_{30}H_{38}O_6$+H, 495.2741).

3.3.5. Ligulasagitin E (5). Optically active oil (11 mg); $[\alpha]_D^{20}$ +35 (*c* 0.5, $CHCl_3$); UV ($CHCl_3$) λ_{max} 314 nm; IR (film) ν_{max} 3418, 2932, 2882, 1706, 1662, 1628, 1366, 1218, 1162, 1090, 1052, 1023 cm^{-1}; ^1H and ^{13}C NMR data: see Table 2; HRESIMS *m/z* 698.3519 $[M+NH_4]^+$ (calcd for $C_{38}H_{48}O_{11}$+NH_4, 698.3535).

3.4. Single-crystal X-ray crystallography of compound 2

Suitable colorless plates were obtained from a solution of acetone. Single-crystal X-ray diffraction study of compound **2** was performed on a BRUKER SMART 1000 CCD diffractometer equipped with a graphite crystal monochromator situated in the incident beam for data collection. Single crystal with dimensions of 0.22×0.20×0.10 mm^3 was chosen for X-ray diffraction studies. The determination of unit cell parameters and data collections were performed with Mo Kα radiation (λ=0.71073 Å) at 294(2) K by using the ω scan mode in the range of $2.60<\theta<25.49°$, with $-8\le h\le 8$, $-5\le k\le 9$, $-18\le l\le 19$. The reflections collected are 4467 and the unique reflections are 2262 [*R*(int)= 0.0341]. The structure was solved by direct method using SHELXS program of the SHELXL-97 package. X-ray data of **2**: $C_{19}H_{26}O_5$, *M*=334.40, monoclinic, dimensions: 0.22× 0.20×0.10 mm, space group *P*21, Mo Kα, final *R* indices [$I>2\sigma(I)$], *R*1=0.0608, *wR*2=0.1452, *a*=6.6703(11), *b*= 8.2171(13), *c*=15.714(3) Å, α=90, β=92.889(2), γ=90°, *V*=860.2(2) $Å^3$, *Z*=2, *d*=1.291 g/cm^3, number of observations [$>2\sigma(I)$] 3546, parameters 223. Crystallographic data for **2** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 654457).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2007.10.001.

References and notes

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