Tetrahedron 71 (2015) 8428–8435

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Terpenoids from *Ligularia virgaurea* collected in China: the first example of two bakkane derivatives with an anhydride-type ring C and nineteen new chemical constituents

Yoshinori Saito [a], Saori Iga [b], Katsuyuki Nakashima [b], Yasuko Okamoto [b], Xun Gong [c,*,‡], Chiaki Kuroda [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] Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, China
[d] Department of Chemistry and Research Center of Smart Molecules, Rikkyo University, Nishi-Ikebukuro, Toshima-ku, Tokyo 171-8501, Japan

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 1 September 2015
Accepted 3 September 2015
Available online 8 September 2015

Keywords:
Asteraceae
Ligularia virgaurea
Terpenoid
Eremophilane
Bakkane
Anhydride
Isolation

ABSTRACT

Further chemical investigation of two *Ligularia virgaurea* samples collected in China resulted in the isolation of 21 new compounds, two of which were bakkane-type sesquiterpenoids bearing an anhydride-type ring C, which was a previously unknown partial structure. These samples belonged to the V-type (the major component was virgaurenone) among the five chemotypes found in this species.

1. Introduction

Ligularia (Asteraceae) is a highly diversified genus in the Hengduan Mountain region of China, and we have expressed the presence of intra-specific diversity in many species in terms of both the chemical composition and DNA sequence of an evolutionarily neutral region.[1] *Ligularia virgaurea* (Maxim.) Mattf. is an abundant species in the western area of the Sichuan province. We previously reported that the species collected in the Sichuan, Qinghai, and Gansu provinces were grouped into the following five chemotypes on the basis of their terpenoid constituents: L-type (the major component was ligularol), V-type (virgaurenone A), C-type (cacalol), H-type (6-hydroxyeuryopsin), and N-type (neoadenostylone).[2–4] The species were grouped into three clades on the basis of their DNA sequences, which were in good agreement with the chemotypes (clade A=L-type, clade B=V- and H-types, clade C=C- and N-types). In addition to furanoeremophilanes, the major components, we previously isolated a variety of new compounds such as seco-eremophilanes, bakkanes, and compounds having further rearranged carbon skeletons from 38 samples of *L. virgaurea*.[3] Various sesquiterpene dimers were also isolated.[3–7] Since then, four new compounds, including rearranged norsesquiterpenes, were characterized from another sample.[8] We have analyzed two more samples (one from northern Sichuan province and the other from southern Gansu province)[3] and 26 compounds were isolated, 21 of which were new. Two of these compounds have a unique structure, a bakkane bearing an anhydride partial structure. In this study, we describe the details of their structure elucidation.

2. Results and discussion

Compound **1** exhibited a quasi-molecular ion peak at *m/z* 361, and its molecular formula was determined to be $C_{20}H_{24}O_6$ by

* Corresponding authors. Tel.: +86 871 5223625 (X.G.); tel./fax: +81 3 3985 2396 (C.K.); tel.: +81 88 602 8464; fax: +81 88 655 3051 (M.T.); e-mail addresses: gongxun@mail.kib.ac.cn (X. Gong), kuroda5000144@rikkyo.ac.jp (C. Kuroda), tori@ph.bunri-u.ac.jp (M. Tori).
† For general information.
‡ For taxonomy.
§ For structure determination.

http://dx.doi.org/10.1016/j.tet.2015.09.011
0040-4020/

HRCIMS and ^{13}C NMR data. An angelate moiety was apparent from chemical shift values of δ 5.61 (1H, qq, J=7.3, 1.5 Hz), 1.87 (3H, dq, J=7.3 and 1.5 Hz), and 1.69 (3H, quintet, J=1.5 Hz) (Table 1) as well as an IR absorption band at 1722 cm^{-1}. The IR absorption at 1850 cm^{-1} indicated the presence of any of an epoxy-lactone, an enol-lactone, or an anhydride. The observed value was at a slightly higher wavenumber than that at which an epoxy- and an enol-lactone have ever been encountered.[2,3,8] The ^{13}C NMR spectrum indicated the presence of five methyl, two methylene, five methine, and eight quaternary carbon atoms including eight sp^2 carbon atoms (four carbonyl and four olefinic carbons). The degree of unsaturation was 9, and therefore, this compound should be tricyclic. The 2D correlations are shown in Fig. 2. The HMBC correlations between H_3-15 and C-3 and 4, between H_3-14 and C-4, 5, 6, and 10, between H-3 and C-2, and between H-9 and C-1 suggested a six-membered carbocycle for ring A. Because H-6 showed a correlation with C-7 (quaternary carbon), H-9 with C-8 (carbonyl), and H-11 with C-7, this compound was deduced to be a bakkane derivative. Both carbons C-8 and 12 were carbonyl groups; therefore, ring C must be an anhydride, as shown in Fig 2. The angeloyloxy group was substituted at C-6 as indicated by the correlation between H-6 and C-1′. The stereochemistry was determined by the NOESY correlations shown in Fig. 2. Because the NOE between H_3-

Table 1
^1H and ^{13}C NMR data of virgaureno-anhydrides A (**1**) and B (**2**)

Positions	**1**[a]		**2**[a]	
	^1H (mult, J in Hz)	^{13}C	^1H (mult, J in Hz)	^{13}C
1	5.63 (d, 2.4)	123.8	5.68 (br s)	124.0
2	—	196.2	—	196.6
3	1.97 (dd, 17.6, 4.4)	41.4	2.00 (dd, 17.9, 3.4)	41.7
	1.83 (dd, 17.6, 13.7)	—	1.77 (dd, 17.9, 13.4)	—
4	1.47 (dqd, 13.7, 6.6, 4.4)	40.0	1.50–1.57 (m)	39.0
5	—	49.1	—	48.4
6	5.06 (s)	79.4	5.01 (s)	81.6
7	—	57.5	—	55.7
8	—	172.5	—	177.3
9	3.24 (dd, 18.8, 2.4)	38.2	2.07 (dd, 18.8, 1.5)	36.6
	1.42 (d, 18.8)	—	2.02 (dd, 18.8, 2.2)	—
10	—	167.0	—	167.2
11	1.81 (q, 7.1)	45.3	2.58 (q, 7.6)	61.2
12	—	170.2	—	81.5
13	1.10 (d, 7.1)	8.3	0.61 (d, 7.6)	14.0
14	1.06 (s)	11.5	0.33 (s)	12.9
15	0.43 (d, 6.6)	15.4	0.41 (d, 6.8)	15.6
1′	—	166.2	—	168.0
2′	—	126.0	1.41 (s)	20.3
3′	5.61 (qq, 7.3, 1.5)	142.9	—	—
	—	15.9	—	—
4′	1.87 (dq, 7.3, 1.5)	20.1	—	—
5′	1.69 (quint, 1.5)	—	—	—

[a] In C_6D_6.

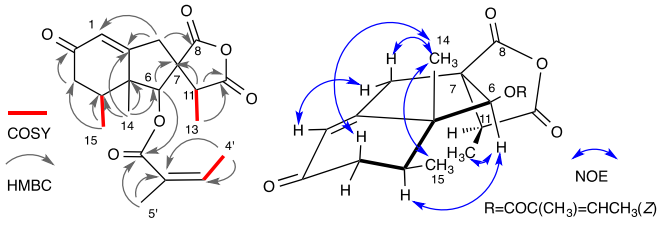

Fig. 2. Selected 2D correlations detected for compound **1**.

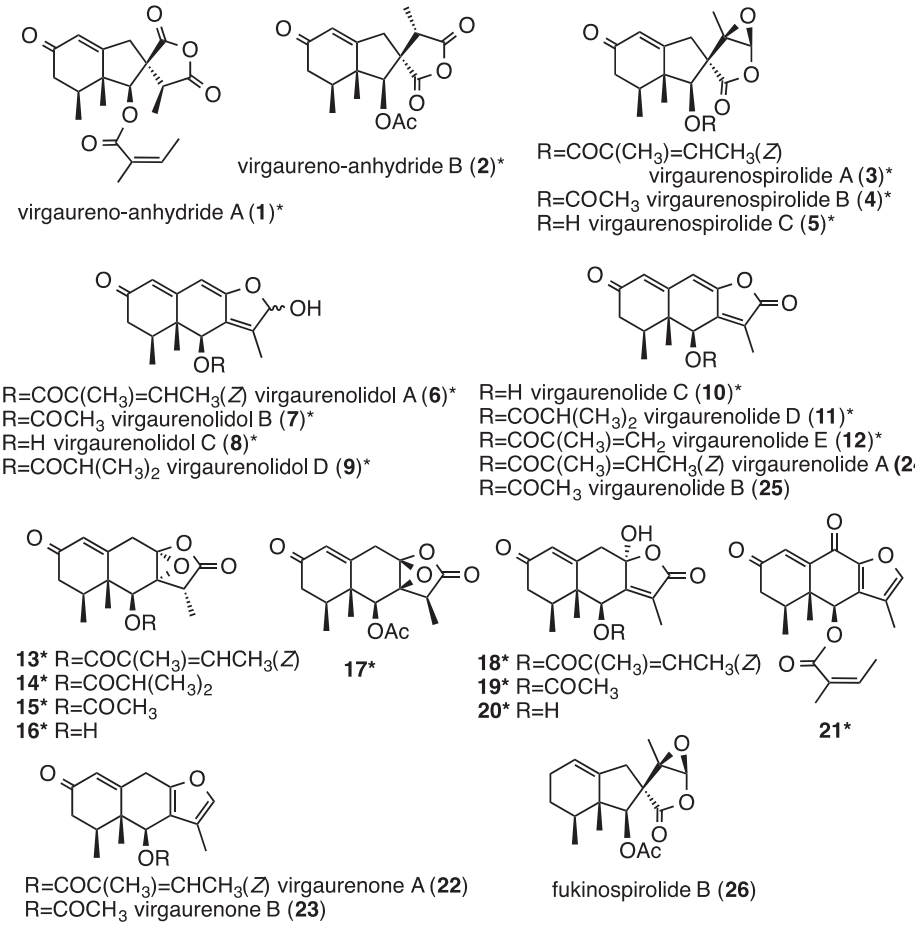

Fig. 1. Compounds isolated in this study (asterisks indicate new compounds).

14 and H-3β was observed, the conformation of ring A should be C-3 up and C-4 down as shown. The NOEs between H-6 and H-4 and between H_3-14 and H_3-15 indicated that both H-6 and H-4 were α. The stereochemistry at C-7 was determined by the NOE between H-6 and H_3-13. Thus, C-11 was established to be down, and the methyl group at C-11 was established to be up (Fig. 2).

This is the first example of a bakkane-type sesquiterpenoid bearing an anhydride partial structure. This is presumably derived from the corresponding epoxide by rearrangement. This compound was named virgaureno-anhydride A.

Compound **2** exhibited similar spectroscopic features to those of compound **1**, except that an angelate moiety was not present and an acetyl methyl signal was observed in this compound (Table 1). The molecular formula was determined to be $C_{17}H_{20}O_6$ by HRCIMS and ^{13}C NMR data. The presence of an anhydride was suggested by the IR absorption bands at 1848 and 1780 cm^{-1}. The 2D correlations are shown in Fig. 3, which were very similar to those of compound **1**. An acetyl group was suggested to be at C-6 due to the correlation between H-6 and C-1′. The difference from compound **1** was the stereochemistry of C-7. The NOE between H_3-13 and H-9β and between H-11 and H_3-14 indicated that the C-8 carbonyl carbon must be down, and the methyl group at C-11 must also be down. The configuration of the methyl group at C-11 needs to be commented on. From the biosynthetic discussion, H_3-13 should be up (vide infra). However, this was established to be down. Therefore, the configuration at C-11 was presumably isomerized by the effect of a bulky substituent present at C-6 close to this methyl group at a later stage of biosynthesis. This compound was named virgaureno-anhydride B.

Compounds **3**–**5** showed similar spectroscopic features to those of compounds **1** and **2** (Table 2). The molecular formulae of compounds **3**, **4**, and **5** were $C_{20}H_{24}O_6$, $C_{17}H_{20}O_6$, and $C_{15}H_{18}O_5$, respectively, based on HRCIMS and ^{13}C NMR data. The differences between these three compounds were the substituents at C-6, namely there is an angelate in **3**, an acetate in **4**, and no acyl group in **5**, as suggested by their ^1H NMR spectra (Table 2) and molecular formulae. The 2D correlations are shown in Fig. 4, and these three compounds had a bakkane skeleton with a 1(10)-en-2-one system. The stereochemistry was suggested by the NOESY correlations to be a C-8 down structure for the spiro carbon at C-7. The epoxide ring must be up because the NOE between H_3-13 and H-9β was observed. Compounds **3**–**5** were named virgaurenospirolides A-C, respectively.

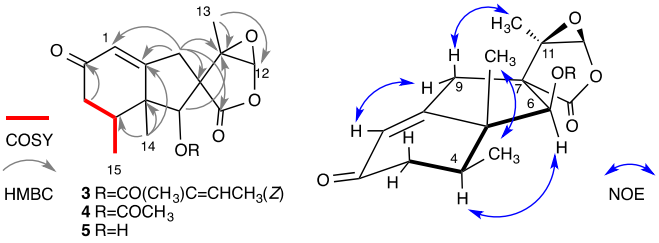

Fig. 4. Selected 2D correlations detected for compounds **3**–**5**.

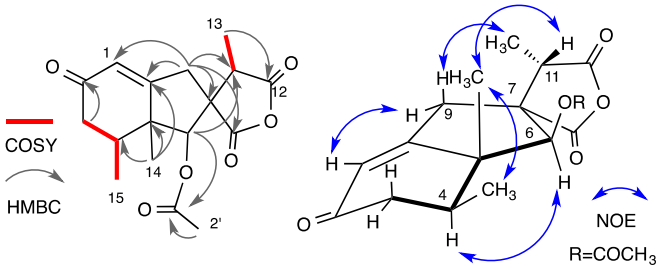

Fig. 3. Selected 2D correlations detected for compound **2**.

Compounds **6**–**9** had similar spectroscopic data to each other. The molecular formulae of compounds **6**–**9** were $C_{20}H_{24}O_5$, $C_{17}H_{20}O_5$, $C_{15}H_{18}O_4$, and $C_{19}H_{24}O_5$, respectively. Seven sp^2 carbon atoms (including one carbonyl) were detected for compound **8**. The degree of unsaturation was 7, and therefore, compound **8** should be tricyclic. The 2D correlations are shown in Fig. 5, indicating the presence of a triene-2-one partial structure substituted with two hydroxy groups at C-6 and 12. Compounds **6**, **7**, and **9** had the same partial structures with esters at C-6 (compound **6** with an angelate, **7** with an acetate, and **9** with an isobutyrate). The stereochemistry of the acyloxy or hydroxy group at C-6 was suggested to be β;

Table 2
^1H and ^{13}C NMR data of virgaurenospirolides A–C, (**3**)–(**5**)

Positions	**3**[a]		**4**[a]		**5**[a]	
	^1H (mult, J in Hz)	^{13}C	^1H (mult, J in Hz)	^{13}C	^1H (mult, J in Hz)	^{13}C
1	5.79 (d, 2.5)	124.1	5.79 (d, 2.4)	124.0	5.80 (d, 2.4)	123.5
2	—	196.6	—	196.6	—	197.0
3	2.05 (dd, 16.6, 3.7)	41.7	2.07 (dd, 16.9, 4.0)	41.7	2.16 (dd, 17.2, 3.6)	41.9
	1.88 (dd, 16.6, 13.2)	—	1.89 (dd, 16.9, 13.2)	—	1.98 (dd, 17.2, 13.6)	—
4	1.94 (dqd, 13.2, 6.6, 3.7)	39.2	1.98 (dqd, 13.2, 6.6, 4.0)	39.0	1.84–1.91 (m)	39.6
5	—	48.6	—	48.4	—	49.0
6	5.724 (s)	81.3	5.58 (s)	81.6	3.74 (d, 3.7)	84.4
7	—	55.6	—	55.7	—	57.0
8	—	177.5	—	177.3	—	178.4
9	2.15 (dd, 17.4, 2.5)	36.8	2.12 (dd, 17.1, 2.4)	36.6	2.09 (dd, 16.9, 2.4)	36.5
	1.796 (d, 17.4)	—	1.74 (d, 17.1)	—	1.68 (d, 16.9)	—
10	—	167.0	—	167.2	—	168.5
11	—	60.9	—	61.2	—	62.8
12	4.40 (s)	81.2	4.42 (s)	81.5	4.46 (s)	81.6
13	0.89 (s)	14.1	0.86 (s)	14.0	0.90 (s)	14.0
14	0.72 (s)	13.0	0.65 (s)	12.9	0.73 (s)	11.8
15	0.55 (d, 6.6)	15.6	0.57 (d, 6.6)	15.6	0.62 (d, 6.6)	15.6
1′	—	164.9	—	168.0	—	—
2′	—	127.0	1.70 (s)	20.3	—	—
3′	5.723 (qq, 7.2, 1.5)	140.4	—	—	—	—
4′	1.97 (dq, 7.2, 1.5)	15.9	—	—	—	—
5′	1.80 (quint, 1.5)	20.6	—	—	—	—

[a] In C_6D_6.

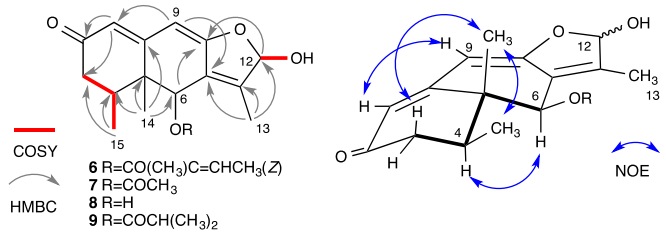

Fig. 5. Selected 2D correlations detected for compounds **6**–**9**.

however, the configuration at C-12 was not determined. Compounds **6**, **7**, and **9** were isolated in only one isomer, being either OH-12 α or β; meanwhile, compound **8** existed in both forms, although the C-12 stereochemistry was unclear (Table 3). Compounds **6**–**9** were named virgaurenolidols A-D, respectively.

respectively by HRCIMS and ^{13}C NMR data. All compounds exhibited an IR absorption band at approximately 1809 cm^{-1} and were deduced to be an epoxy- or an enol-lactone,[2,3,8] which was supported by the presence of quartet signals at δ 2.49–2.83 (*J*=7.1–7.3 Hz) assignable to H-11 in the ^1H NMR spectra (Table 5). The 2D correlations shown in Fig. 7 indicated that these compounds were epoxy-lactones with 1(10)-en-2-one structures. The oxygen functional groups substituted at C-6 were an angeloyloxy, an isobutyroyloxy, an acetoxy, and a hydroxy group for compounds **13**–**16**, respectively. The stereochemistry was established as shown in Fig. 7 by the NOE between H-6α and H-4α and between H-6α and H_3-13 in each compound. If the epoxide ring was β, these NOEs could not feasibly to be detected.[3] In the case of compound **17**, the molecular formula was the same as that of compound **15**, and the spectroscopic features were very similar to those of compound

Table 3
^1H and ^{13}C NMR data of virgaurenolidols A–D, (**6**)–(**9**)

Positions	**6**[a]		**7**[a]		**8**[b]			**9**[a]	
	^1H (mult, *J* in Hz)	^{13}C	^1H (mult, *J* in Hz)	^{13}C	^1H (mult, *J* in Hz)	^{13}C (major)	^{13}C (minor)	^1H (mult, *J* in Hz)	^{13}C
1	5.80 (s)	121.8	5.76 (s)	121.8	5.70 (br s)	121.2	121.5	5.77 (s)	121.9
2	—	199.2	—	199.0	—	201.1	201.1	—	198.7
3	2.32 (dd, 17.1, 4.1)	43.5	2.32 (dd, 16.9, 4.2)	43.4	2.34 (dd, 16.6, 13.0)	44.3	44.3	2.31 (dd, 16.9, 4.0)	43.5
	2.05 (dd, 17.1, 13.7)	—	1.98 (dd, 16.9, 13.4)	—	2.18 (dd, 16.6, 3.2)	—	—	2.01 (dd, 16.9, 13.5)	—
4	2.57–2.64 (m)	39.0	2.57 (dqd, 13.4, 6.6, 4.2)	38.5	2.25–2.30 (m)	40.5	40.4	2.46 (dqd, 13.5, 6.8, 4.0)	39.2
5	—	43.5	—	43.3	—	45.8	46.0	—	43.6
6	5.98 (q, 1.7)	74.7	5.77 (q, 1.9)	75.7	4.63 (br s)	75.4	75.5	5.84 (q, 2.0)	75.3
7	—	126.8	—	126.7	—	131.9	131.9	—	126.8
8	—	164.2	—	164.0	—	166.4	166.6	—	163.8
9	5.43 (s)	97.5	5.34 (s)	97.5	5.48 (br s), 5.46 (br s)	97.1	96.8	5.38 (s)	97.6
	—	—	—	—	—	—	—	—	—
10	—	165.2	—	164.6	—	171.0	171.5	—	164.7
11	—	143.2	—	142.8	—	145.3	144.5	—	143.1
12	6.00 (br s)	108.8	5.99 (d, 12.4)	108.9	6.05 (br s)	108.1	108.8	5.95 (d, 12.4)	108.5
13	1.68 (br s)	10.1	1.62 (br s)	10.0	2.09 (br s)	11.0	11.1	1.63 (br s)	10.5
14	1.00 (s)	14.1	0.92 (s)	13.6	1.10 (br s), 1.16 (br s)	13.5	13.5	0.943 (s)	14.0
15	0.90 (d, 6.6)	17.8	0.86 (d, 6.6)	18.0	1.26 (d, 6.4)	18.7	18.7	0.84 (d, 6.8)	18.0
1′	—	166.7	—	170.1	—	—	—	—	175.8
2′	—	127.0	1.53 (s)	20.7	—	—	—	2.20 (sept, 7.1)	84.6
3′	5.73 (br q, 7.3)	142.0	—	—	—	—	—	0.944 (d, 7.1)	18.5
4′	1.88 (dq, 7.3, 1.5)	16.0	—	—	—	—	—	0.940 (d, 7.1)	18.5
5′	1.73 (quint, 1.5)	20.3	—	—	—	—	—	—	—
OH	7.38 (br s)	—	7.34 (d, 12.4)	—	—	—	—	6.96 (d, 12.4)	—

[a] In C_6D_6.
[b] In CD_3OD.

Compounds **10**–**12** were derivatives of virgaurenolides, with all of them having the same skeleton of a triene-2,12-dione structure with a different acyloxy or hydroxy group at C-6 (Fig. 6 and Table 4). Compounds **11** and **12** were isolated in almost a 1:1 mixture and analyzed without further separation. The stereochemistry at C-6 was established by the NOE between H-6α and H-4α and between H_3-14 and H_3-15 (Fig. 6). Compounds **10**–**12** were named virgaurenolides C-E, respectively.

The molecular formulae of compounds **13**–**16** were determined to be $C_{20}H_{24}O_6$, $C_{19}H_{24}O_6$, $C_{17}H_{20}O_6$, and $C_{15}H_{18}O_5$,

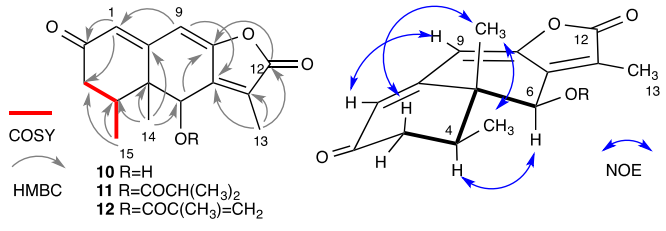

Fig. 6. Selected 2D correlations detected for compounds **10**–**12**.

15. The 2D correlations shown in Fig. 8 indicated that this compound had the same planar structure as that of **15**. However, the quartet proton was detected at δ 2.38 (*J*=7.1 Hz, H-11), which was slightly higher field than for **15** (Table 5). The configuration at C-11 was established by the NOESY correlations between H-6α and H-4α and between H-6α and H-11α. Therefore, compound **17** was established to be 11αH-7β,8β-epoxy-2-oxoeremophil-1(10)-en-12,8-olide.

Three compounds, **18**–**20**, had molecular formulae of $C_{20}H_{24}O_6$, $C_{17}H_{20}O_6$, and $C_{15}H_{18}O_5$, respectively. The ^1H and ^{13}C NMR data indicated that these three had a 1(10)-en-2-one system (Table 6) and a γ-lactone (see Experimental). The 2D correlations for these compounds suggested that they had an eremophilenolide skeleton with a 1(10)-en-2-one partial structure and oxygen functional groups at C-6 and 8 (Fig. 9). The stereochemistry was determined by the NOE between H_3-14 and H-9β and between H-6α and H-4α, indicating that the hydroxy group at C-8 should be α. If this group was β, the conformations of rings A and B should be different from those observed, as previously discussed.[2,3,8]

Table 4
^{1}H and ^{13}C NMR data of virgaurenolides C–E, (**10**)–(**12**)

Positions	**10**[a]		**11**[a]		**12**[a]	
	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C
1	5.74 (s)	128.8	5.72 (s)	129.0	5.73 (s)	129.1
2	—	195.9	—	195.4	—	195.4
3	2.13 (dd, 17.4, 4.2)	43.6	2.03 (dd, 17.6, 4.4)	43.5	2.01 (dd, 17.3, 4.4)	43.5
	1.96 (dd, 17.4, 13.6)	—	1.92 (dd, 17.6, 13.6)	—	1.90 (dd, 17.3, 13.5)	—
4	1.67 (dqd, 13.6, 6.6, 4.2)	38.9	1.69–1.78 (m)	38.0	1.69–1.78 (m)	38.0
5	—	45.0	—	44.3	—	44.3
6	3.75 (dq, 8.8, 1.9)	74.5	5.70 (q, 1.7)	73.6	5.78 (q, 2.0)	74.0
7	—	146.6	—	143.6	—	143.6
8	—	151.5	—	151.2	—	151.2
9	5.28 (s)	106.8	5.29 (s)	107.2	5.29 (s)	107.1
	—	—	—	—	—	—
10	—	158.6	—	157.5	—	157.2
11	—	126.2	—	125.7	—	125.7
12	—	169.5	—	169.1	—	169.1
13	1.76 (d, 1.9)	9.4	1.54 (d, 1.7)	8.9	1.54 (d, 2.0)	8.5
14	0.53 (s)	12.1	0.72 (s)	13.7	0.72 (s)	13.5
15	0.73 (d, 6.6)	17.9	0.52 (d, 6.6)	17.1	0.54 (d, 6.6)	16.9
1′	—	—	—	175.2	—	165.9
2′	—	—	2.14 (sept, 7.1)	34.4	—	135.5
3′	—	—	0.93 (d, 7.1)	18.5	5.15 (quint, 1.4)	127.5
	—	—	—	—	5.94 (br s)	—
4′	—	—	0.93 (d, 7.1)	18.5	1.69 (br s)	18.0
OH	0.80 (d, 8.8)	—	—	—	—	—

[a] In C_6D_6.

Table 5
^{1}H and ^{13}C NMR data of compounds **13**–**17**

Positions	**13**[a]		**14**[a]		**15**[a]		**16**[a]		**17**[a]	
	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C
1	5.70 (s)	130.5	5.68 (d, 2.0)	130.5	5.68 (d, 1.9)	130.5	5.71 (d, 2.0)	129.6	5.52 (d, 2.4)	129.6
2	—	195.4	—	195.2	—	195.3	—	196.1	—	195.4
3	2.45 (dd, 16.2, 3.9)	42.1	2.41 (dd, 16.1, 4.9)	42.0	2.40 (dd, 16.2, 4.6)	42.0	2.14 (dd, 15.9, 4.9)	42.2	2.07 (dd, 16.9, 4.1)	42.4
	1.89 (dd, 16.2, 4.3)	—	1.91 (dd, 16.1, 4.2)	—	1.91 (dd, 16.2, 3.4)	—	1.95 (dd, 15.9, 4.2)	—	1.88 (dd, 16.9, 11.4)	—
4	1.56–1.61 (m)	33.7	1.53–1.58 (m)	33.7	1.48–1.54 (m)	33.6	1.59–1.64 (m)	33.3	1.66 (dqd, 11.4, 6.9, 4.1)	38.8
5	—	43.1	—	43.1	—	42.9	—	44.0	—	43.2
6	5.46 (s)	69.8	5.34 (s)	69.6	5.29 (s)	70.1	3.39 (d, 6.6)	70.3	5.00 (s)	73.7
7	—	65.3	—	65.2	—	65.1	—	66.6	—	64.7
8	—	86.5	—	86.6	—	86.5	—	86.8	—	86.5
9	2.45 (d, 14.9)	—	2.44 (dd, 14.9, 2.0)	31.3	2.43 (dd, 15.2, 1.9)	31.3	2.44 (dd, 15.2, 2.0)	31.5	2.66 (dd, 18.1, 2.4)	29.8
	2.35 (d, 14.9)		2.33 (d, 14.9)	—	2.33 (d, 15.2)	—	2.34 (d, 15.2)	—	2.25 (d, 18.1)	—
10	—	151.3	—	151.2	—	151.3	—	154.3	—	156.3
11	2.68 (q, 7.3)	40.5	2.60 (q, 7.3)	40.1	2.49 (q, 7.3)	40.1	2.83 (q, 7.1)	40.1	2.38 (q, 7.1)	42.1
12	—	174.6	—	174.7	—	174.6	—	175.3	—	174.8
13	1.18 (d, 7.3)	10.9	1.14 (d, 7.3)	11.0	1.10 (d, 7.3)	10.9	1.29 (d, 7.1)	11.3	1.12 (d, 7.1)	11.5
14	0.52 (s)	17.1	0.51 (s)	17.0	0.49 (s)	16.8	0.49 (s)	15.8	0.89 (s)	13.4
15	0.49 (d, 7.1)	15.3	0.52 (d, 7.4)	15.3	0.52 (d, 6.9)	15.3	0.61 (d, 6.9)	15.7	0.52 (d, 6.9)	16.4
1′	—	165.4	—	174.6	—	168.7	—	—	—	169.8
2′	—	126.1	2.12 (sept, 7.1)	34.1	1.48 (s)	19.8	—	—	1.50 (s)	20.3
3′	5.71 (q, 7.3)	142.3	0.87 (d, 7.1)	18.9	—	—	—	—	—	—
4′	1.85 (dq, 7.3, 1.4)	16.0	—	18.6	—	—	—	—	—	—
5′	1.60 (quint, 1.4)	20.4	0.86 (d, 7.1)	—	—	—	—	—	—	—
OH	—	—	—	—	—	—	1.44 (d, 6.6)	—	—	—

[a] In C_6D_6.

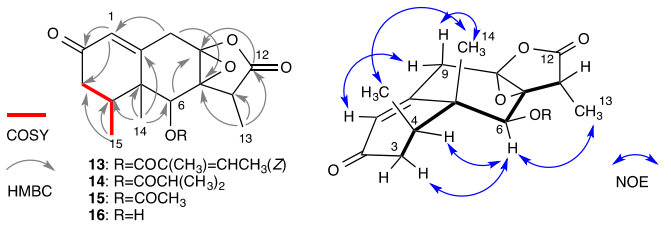

Fig. 7. Selected 2D correlations detected for compounds **13**–**16**.

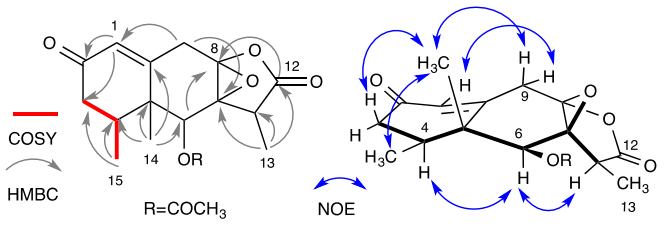

Fig. 8. Selected 2D correlations detected for compound **17**.

Table 6
^{1}H and ^{13}C NMR data of compounds **18**–**21**

Positions	**18**[a]		**19**[a]		**20**[a]		**21**[a]	
	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C	^{1}H (mult, *J* in Hz)	^{13}C
1	5.92 (d, 2.0)	130.1	5.78 (d, 2.0)	130.3	5.94 (d, 1.7)	129.4	7.08 (s)	129.8
2	—	197.3	—	196.1	—	199.1	—	196.8
3	2.01 (dd, 16.6, 3.9)	43.0	2.00 (dd, 16.9, 4.0)	43.0	2.17 (dd, 15.2, 2.5)	43.4	1.99 (dd, 15.7, 2.2)	43.3
	1.86 (dd, 16.6, 12.4)	—	1.79 (dd, 16.9, 12.4)	—	1.97 (dd, 15.2, 12.2)	—	1.83 (dd, 15.7, 13.0)	—
4	1.65 (dqd, 12.4, 6.6, 3.9)	38.0	1.51–1.58 (m)	37.5	2.02 (dqd, 12.2, 6.4, 2.5)	38.3	1.86–1.92 (m)	38.5
5	—	46.9	—	46.6	—	48.1	—	47.2
6	5.74 (q, 1.7)	76.9	5.48 (q, 1.7)	77.0	4.54 (q, 1.7)	76.3	6.11 (s)	72.1
7	—	154.3	—	153.7	—	158.8	—	136.4
8	—	102.0	—	101.2	—	101.9	—	147.1
9	2.89 (d, 14.4)	45.0	2.55 (d, 14.4)	44.6	2.84 (d, 14.4)	45.2	—	173.1
	2.20 (dd, 14.4, 2.0)	—	2.02 (dd, 14.4, 2.0)	—	2.29 (dd, 14.4, 1.7)	—	—	—
10	—	158.7	—	156.8	—	161.0	—	155.8
11	—	124.2	—	124.6	—	124.7	—	121.3
12	—	171.2	—	170.3	—	172.5	6.63 (q, 1.0)	147.1
13	1.72 (d, 1.7)	7.9	1.65 (d, 1.7)	8.1	2.13 (d, 1.7)	8.8	1.53 (d, 1.0)	8.3
14	0.64 (s)	12.7	0.48 (s)	12.1	0.71 (s)	11.5	0.78 (s)	14.2
15	0.61 (d, 6.6)	17.5	0.55 (d, 6.9)	17.4	1.02 (d, 6.4)	18.2	0.58 (d, 6.4)	16.7
1′	—	166.2	—	169.3	—	—	—	166.4
2′	—	126.3	1.52 (s)	20.3	—	—	—	126.8
3′	5.75 (qq, 7.3, 1.5)	143.1	—	—	—	—	5.76 (qq, 7.3, 1.5)	142.2
4′	1.87 (dq, 7.3, 1.5)	16.0	—	—	—	—	1.94 (dq, 7.3, 1.5)	16.0
5′	1.68 (quint, 1.5)	20.4	—	—	—	—	1.71 (quint, 1.5)	20.4

[a] In C_6D_6.

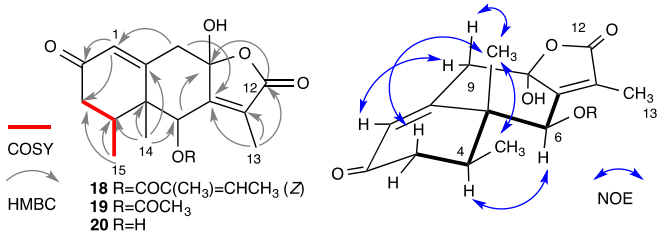

Fig. 9. Selected 2D correlations detected for compounds **18**–**20**.

The IR spectrum of the final compound **21** showed absorption bands at 1720 and 1672 cm^{-1}. The ^{13}C NMR data exhibited the presence of five methyl, one methylene, five methine, and nine quaternary carbon atoms, including three carbonyl and eight olefinic carbons. The molecular formula was $C_{20}H_{22}O_5$ (by HRMS and ^{13}C NMR), and therefore, this was determined to be a tricyclic compound. The ^{1}H and ^{13}C NMR data suggested the presence of a furan (Table 6), and a furanoeremophilane skeleton was shown by the 2D correlations (Fig. 10). One carbonyl (δ 196.3) was placed at C-2, and the other one (δ 173.1) was not indicated by the HMBC correlations. However, the position of this carbonyl group at C-9 was best explained based on the spectroscopic data. A similar discussion was reported for all of the 9-oxofuranoeremophilane compounds ever isolated.[9–12] The angeloyloxy group was substituted at C-6 as indicated by the chemical shift (δ_H 6.11, δ_C 72.1) (Table 6). The NOE between H-6α and H-4α indicated the stereochemistry for this compound.

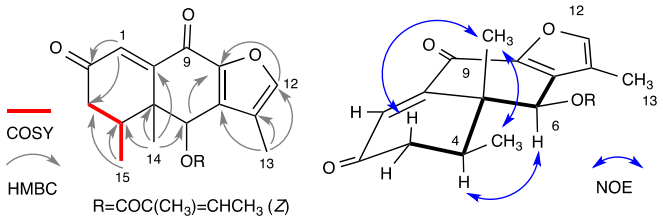

Fig. 10. Selected 2D correlations detected for compound **21**.

The absolute configurations of the compounds isolated in this work were studied by CD spectra. Most of the compounds had a 1(10)-en-2-one system, with some of them having a 1(10),7(11),8-triene-2,12-dione or a 1(10),7(11),8-triene-2-one system. The CD spectra of compounds with such a system resembled each other, namely, a positive Cotton effect at approximately 240–250 nm and a negative Cotton effect at approximately 300–340 nm for a 1(10)-en-2-one system (see Experimental). The enone chirality rule[13] was not easy to apply for these compounds because the distortion of the carbonyl and the olefin was not sufficiently large to predict the sign of the Cotton effect. Therefore, DFT calculations were carried out for compound **5**. The results of the calculations and the experimental CD spectra are shown in Fig. 11. These results were quite similar to each other, and the absolute configuration of compound **5** was established as depicted in the formula. Hence, other compounds isolated here were deduced to have the same absolute configuration as that of compound **5**.

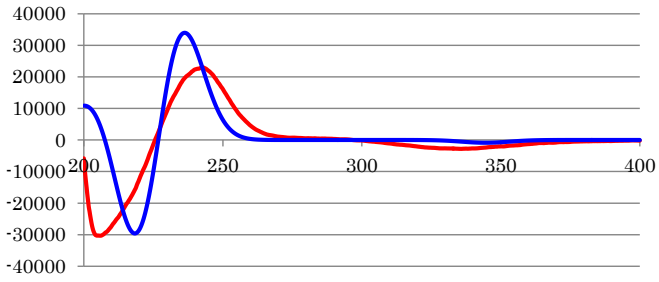

Fig. 11. The CD spectra of compound **5** (red: observed; blue: calculated).

Other compounds were virgaurenone A (**22**), virgaurenone B (**23**), virgaurenolide A (**24**), virgaurenolide B (**25**), and fukinospirolide B (**26**) (Fig. 1). The major constituent of sample 1 (2010–63) was virgaurenone B (**23**) (10.1% of the extract), and that of sample 2 (2010–68) was virgaurenolidol A (**6**) (6.1% of the extract). As previously reported,[3] these two samples belong to the V-type (a group consisting of virgaurenone and virgaurenolide derivatives). Virgaureno-anhydrides, **1** and **2**, were new type of compounds, which had a cyclic anhydride moiety, a hitherto unknown partial

structure in the bakkane skeleton. Lactols have been isolated from *Ligularia* plants, two precedents of which had oxygen functional groups both at C-8 and C-12.[14,15] Virgaurenolidols, **6**–**9**, correspond to a reduced-type of virgaurenolide.

Finally, it might be worth discussing plausible biosynthetic pathways. Plausible biosynthetic pathways of bakkane-type sesquiterpenoids have been previously mentioned.[3] Anhydride-type compounds may be produced by ring opening of an epoxide ring (rearrangement) leading to **1** from **27** (Fig. 12). However, in the case of virgaureno-anhydride B (**2**), when the epoxide ring of compound **4** rearranged, H_3-13 should be up because the hydride attacked from the backside at C-11 resulting in H_3-13 being up. Therefore, the configuration at C-11 could best be explained by isomerization at a later stage of biosynthesis due to a bulky substituent at C-6 close to this position (vide supra).

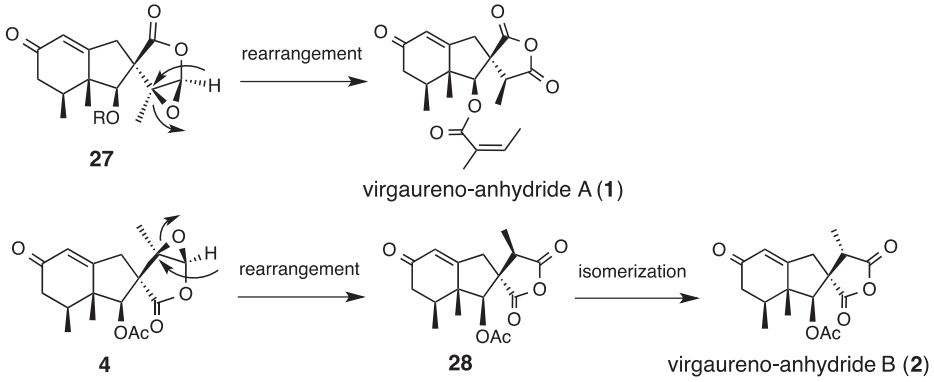

Fig. 12. Plausible biosynthetic pathways for virgaureno-anhydrides A (**1**) and B (**2**).

3. Conclusions

We have isolated 26 compounds from two species of *L. virgaurea* collected in China (one from northern Sichuan province and the other from southern Gansu province). New compounds included three virgaurenospirolides, **3**–**5**, four virgaurenolidols A–D, **6**–**9**, three virgaurenolides C–E, **10**–**12**, five epoxy-lactones, **13**–**17**, three lactones, **18**–**20**, an enedione **21**, and two anhydrides, **1** and **2**. The structures of virgaureno-anhydrides, **1** and **2**, were especially novel. All of the compounds isolated had a 1(10)-en-2-one system, and this partial structure was recognized as characteristic to this chemotype (V-type) of *L. virgaurea*. Absolute configurations were determined by the CD spectra.

4. Experimental

4.1. General

Specific rotations and CD spectra were measured on JASCO DIP-1030 and JASCO J-725 auto recording polarimeters; IR spectra on a SHIMADZU FT/IR-8400S spectrophotometer (Diffuse reflection method on KBr); ^{1}H and ^{13}C NMR spectra on Varian Unity 600 (600 MHz and 150 MHz, respectively), Varian 500-MR (500 MHz and 125 MHz, respectively), and JEOL ECP 400 (400 MHz and 100 MHz, respectively) spectrometers. Mass spectra, including high-resolution spectra, were recorded on a JEOL JMS-700 MStation. Chemcopak Nucleosil 50–5 (4.6×250 mm) and TSK-GEL G1000H_{HR} (7.8×300 mm) were used for HPLC with a solvent system of hexane–ethyl acetate. Silica gel 60 (70–230 mesh, Fuji Sylisia), Wakogel C-200 or Wakogel C-300 (Wako) was used for column chromatography. Silica gel 60 F_{254} plates (Merck) were used for TLC. Ehrlich's test was carried out as previously described.[2]

4.2. Plant materials

See Ref. 3: sample 1 corresponds to that of 20 (in Table 1 of Ref. 3; northern Sichuan province) and sample 2 to 37 (southern Gansu province).

4.3. Extraction and purification for the structural determination

The root of sample 1 (2010–15) (dry weight 7.6 g) was cut into pieces and extracted with EtOAc to afford an extract (554 mg). The residue was subjected to silica-gel column chromatography (hexane–EtOAc, gradient), followed by HPLC (Nucleosil 50-5 and TSK-GEL G1000H_{HR}; hexane–EtOAc) to give **2** (1.5 mg), **4** (3.7 mg), **5** (0.5 mg), **7** (2.3 mg), **10** (0.7 mg), **13** (1.3 mg), **15** (8.7 mg), **16** (0.8 mg), **17** (0.9 mg), **19** (2.7 mg), **22** (6.1 mg), **23** (54.5 mg), **25** (1.4 mg), and **26** (0.6 mg).

The root of sample 2 (2010–68) (dry weight 3.1 g) was cut into pieces and was extracted with EtOAc to afford an extract (405 mg). The residue was subjected to silica-gel column chromatography (hexane–EtOAc, gradient), followed by HPLC (Nucleosil 50-5 and TSK-GEL G1000H_{HR}; hexane–EtOAc) to give **1** (1.4 mg), **3** (3.8 mg), **5** (1.5 mg), **6** (24.7 mg), **7** (0.7 mg), **8** (4.5 mg), **9** (2.2 mg), **10** (2.3 mg), **11** and **12** (0.6 mg; 1:1), **13** (7.7 mg), **14** (0.5 mg), **16** (4.5 mg), **18** (6.6 mg), **20** (1.6 mg), **21** (0.9 mg), and **24** (4.7 mg).

4.4. Compound data

1: $[\alpha]_D^{21}$ −50.0 (0.10, EtOH); FTIR 1850, 1788, 1722, 1666 cm^{-1}; MS (CI) *m*/*z* 361 $[M+H]^+$, 333, 261, 83 (base); HRMS (CI) Obs. *m*/*z* 361.1657 $[M+H]^+$ (Calcd for $C_{20}H_{25}O_6$ 361.1651); CD [θ] −40,000 (205 nm), +21,000 (234), −4300 (327).

2: $[\alpha]_D^{21}$ −13.0 (0.12, EtOH); FTIR 1848, 1780, 1735, 1655 cm^{-1}; MS (CI) *m*/*z* 321 $[M+H]^+$, 293, 279 (base), 233; HRMS (CI) Obs. *m*/*z* 321.1346 $[M+H]^+$ (Calcd for $C_{17}H_{21}O_6$ 321.1338); CD [θ] −2600 (226 nm), -1400 (201), +1200 (242), −100 (330).

3: $[\alpha]_D^{21}$ +17.7 (0.15, EtOH); FTIR 1782, 1726, 1715, 1666 cm^{-1}; MS (CI) *m*/*z* 361 $[M+H]^+$ (base), 349, 333, 279, 233, 83; HRMS (CI) Obs. *m*/*z* 361.1651 $[M+H]^+$ (Calcd for $C_{20}H_{25}O_6$ 361.1651); CD [θ] −31,000 (204 nm), +39,000 (236), −2000 (358).

4: $[\alpha]_D^{21}$ +3.2 (0.37, EtOH); FTIR 1786, 1747, 1663 cm^{-1}; MS (CI) *m*/*z* 321 $[M+H]^+$ (base), 279, 278, 233, 191; HRMS (CI) Obs. *m*/*z* 321.1336 $[M+H]^+$ (Calcd for $C_{17}H_{21}O_6$ 321.1338); CD [θ] −17,000 (203 nm), +22,000 (237), −1700 (344).

5: $[\alpha]_D^{22}$ +13.8 (0.05, EtOH); FTIR 3442, 1788, 1730, 1649 cm^{-1}; MS (CI) *m*/*z* 279 $[M+H]^+$ (base), 261, 233; HRMS (CI) Obs. *m*/*z*

279.1223 $[M+H]^+$ (Calcd for $C_{15}H_{19}O_5$ 279.1233); CD [θ] −30,000 (206 nm), +23,000 (243), −2800 (336).

6: $[\alpha]_D^{21}$ −339 (0.05, EtOH); FTIR 3300, 1715, 1614 cm^{-1}; MS (CI) *m/z* 345 $[M+H]^+$ (base), 327, 245, 229; HRMS (CI) Obs. *m/z* 345.1703 $[M+H]^+$ (Calcd for $C_{20}H_{25}O_5$ 345.1702); CD [θ] +25,000 (201 nm), +8100 (246), +2200 (266), +19,000 (317), −45,000 (363).

7: $[\alpha]_D^{22}$ −267 (0.23, EtOH); FTIR 3242, 1747, 1614, 1568 cm^{-1}; MS (CI) *m/z* 305 $[M+H]^+$ (base), 304, 245, 229; HRMS (CI) obs. *m/z* 305.1389 $[M+H]^+$, calcd for $C_{17}H_{21}O_5$ 305.1389; CD [θ] −2600 (226 nm), +3100 (249), +12,000 (318), −28,000 (364).

8: $[\alpha]_D^{21}$ −534 (0.27, EtOH); FTIR 3275, 1605,1555 cm^{-1}; MS (CI) *m/z* 263 $[M+H]^+$ (100), 262, 245; HRMS (CI) Obs. *m/z* 263.1278 $[M+H]^+$ (Calcd for $C_{15}H_{19}O_4$ 263.1283); CD [θ] +62,000 (202 nm), −13,000 (228), +6700 (253), +19,000 (317), −60,000 (362).

9: $[\alpha]_D^{21}$ −417 (0.2, EtOH); FTIR 3219, 1732, 1605, 1566 cm^{-1}; MS (CI) *m/z* 333 $[M+H]^+$ (base), 329, 315, 273, 261, 245; HRMS (CI) Obs. *m/z* 333.1698 $[M+H]^+$ (Calcd for $C_{19}H_{25}O_5$ 333.1702); CD [θ] +38,000 (201 nm), −5600 (225), +7000 (248), +20,000 (318), −52,000 (365).

10: $[\alpha]_D^{22}$ −104 (0.072, EtOH); FTIR 1782, 1744, 1665, 1632 cm^{-1}; MS (CI) *m/z* 261 $[M+H]^+$ (base), 260, 245, 227, 203; HRMS (CI) obs. *m/z* 261.1131 $[M+H]^+$, calcd for $C_{15}H_{17}O_4$ 261.1127; CD [θ] −11,000 (202 nm), −800 (251), +750 (277), −4600 (363).

11, **12** mixture: $[\alpha]_D^{21}$ −458 (0.056, EtOH); FTIR 1778, 1738, 1668, 1643, 1582 cm^{-1}; MS (CI) *m/z* 331 $[M+H]^+$ (base), 329 $[M+H]^+$, 261, 243; HRMS (CI) Obs. *m/z* 331.1538 $[M+H]^+$ (Calcd for $C_{19}H_{23}O_5$ 331.1545 (for **11**) and *m/z* 329.1396 [M+H]+, calcd for $C_{19}H_{21}O_5$ 329.1389 (for **12**); CD [θ] +52,000 (202 nm), −2900 (228), −27,000 (363).

13: $[\alpha]_D^{21}$ −7.2 (0.12, EtOH); FTIR 1809, 1730, 1682 cm^{-1}; MS (CI) *m/z* 361 $[M+H]^+$, 360, 305, 278, 261, 245, 83 (base); HRMS (CI) Obs. *m/z* 361.1647 $[M+H]^+$ (Calcd for $C_{20}H_{25}O_6$ 361.1651); CD [θ] −6700 (204 nm), +14,000 (232).

14: $[\alpha]_D^{20}$ −40.4 (0.03, EtOH); FTIR 1809, 1742, 1682 cm^{-1}; MS (FAB) *m/z* 349 $[M+H]^+$ (base), 279, 278, 261, 233; HRMS (FAB) Obs. *m/z* 349.1642 $[M+H]^+$ (Calcd for $C_{19}H_{25}O_6$ 349.1651); CD [θ] −44,000 (201 nm), +22,000 (240), −5200 (362).

15: $[\alpha]_D^{16}$ +3.78 (0.23, EtOH); FTIR 1809, 1755, 1732, 1682 cm^{-1}; MS (CI) *m/z* 321 $[M+H]^+$, 303, 278 (100), 261; HRMS (CI) Obs. *m/z* 321.1330 $[M+H]^+$ (Calcd for $C_{17}H_{21}O_6$ 321.1338); CD [θ] −20,000 (207 nm), +49,000 (233 nm), −1400 (337).

16: $[\alpha]_D^{21}$ +9.9 (0.003, EtOH); FTIR 3346, 1807, 1678, 1666 cm^{-1}; MS (CI) *m/z* 279 $[M+H]^+$ (base), 261, 233; HRMS (CI) Obs. *m/z* 279.1231 $[M+H]^+$ (Calcd for $C_{15}H_{19}O_5$ 279.1232); CD [θ] −33,000 (206 nm), +30,000 (246), −2200 (340).

17: $[\alpha]_D^{21}$ −17.3 (0.09, EtOH); FTIR 1807, 1744, 1670 cm^{-1}; MS (FAB) *m/z* 321 $[M+H]^+$ (base), 303, 279, 261, 233; HRMS (FAB) Obs. *m/z* 321.1314 $[M+H]^+$ (Calcd for $C_{17}H_{21}O_6$ 321.1338); CD [θ] −3300 (202 nm), +1500 (235), +430 (285), −200 (360).

18: $[\alpha]_D^{20}$ −121.1 (0.06, EtOH); FTIR 3296, 1768, 1722, 1672 cm^{-1}; MS (FAB) *m/z* 361 $[M+H]^+$, 307, 289, 176, 154 (base), 136; HRMS (FAB) Obs. *m/z* 361.1651 $[M+H]^+$ (Calcd for $C_{20}H_{25}O_6$ 361.1651); CD [θ] −28,000 (206 nm), +18,000 (236), −11,000 (255), +5100 (316).

19: $[\alpha]_D^{23}$ −35.6 (0.22, EtOH); FTIR 3356, 1747, 1664 cm^{-1}; MS (FAB) *m/z* 321 $[M+H]^+$, 303 (base), 302; HRMS (FAB) Obs. *m/z* 321.1330 $[M+H]^+$ (Calcd for $C_{17}H_{21}O_6$ 321.1338); CD [θ] −16,000 (204 nm), +11,000 (234), −3700 (255), +2100 (319).

20: $[\alpha]_D^{20}$ −89.1 (0.13, EtOH); FTIR 3358, 1745, 1666 cm^{-1}; MS (FAB) *m/z* 279 $[M+H]^+$, 261, 173, 154 (base), 136; HRMS (FAB) Obs. *m/z* 279.1227 $[M+H]^+$ (Calcd for $C_{15}H_{19}O_5$ 279.1232); CD [θ] −25,000 (217 nm), +22,000 (240), −3000 (261), −5200 (362).

21: $[\alpha]_D^{21}$ −78.8 (0.05, EtOH); FTIR 1720, 1672 cm^{-1}; MS (CI) *m/z* 343 $[M+H]^+$ (base), 342, 243, 242, 83; HRMS (CI) Obs. *m/z* 343.1547 $[M+H]^+$ (Calcd for $C_{20}H_{23}O_5$ 343.1545); CD [θ] −13,000 (230 nm), −25,000 (258), +10,000 (317), −5100 (380).

Acknowledgements

The authors thank Prof. Xiaojiang Hao and Mrs. Guowen Hu of Kunming Institute of Botany for the international research co-ordination. This work was partly supported by a Grant-in-Aid for Scientific Research from JSPS (No. 25303010).

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.09.011. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Kuroda, C.; Hanai, R.; Nagano, H.; Tori, M.; Gong, X. *Nat. Prod. Commun.* **2012**, *7*, 539–548.
2. 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. Saito, Y.; Takashima, Y.; Kamada, A.; Suzuki, Y.; Suenaga, M.; Okamoto, Y.; Matsunaga, Y.; Hanai, R.; Kuroda, C.; Gong, X.; Tori, M. *Tetrahedron* **2012**, *68*, 10011–10029.
4. Liu, X.; Wu, Q.-X.; Wei, X.-N.; Shi, Y.-P. *Helv. Chim. Acta* **2007**, *90*, 1802–1810.
5. Wu, Q.-X.; Yang, A.-M.; Shi, Y.-P. *Tetrahedron* **2005**, *61*, 10529–10535.
6. Wang, B.-G.; Jia, Z.-J.; Yang, X.-P. *Planta Med.* **1997**, *63*, 577–578.
7. Sun, X.-B.; Xu, Y.-J.; Qui, D.-F.; Yuan, C.-S. *Helv. Chim. Acta* **2007**, *90*, 1705–1711.
8. Saito, Y.; Taniguchi, M.; Komiyama, T.; Ohsaki, A.; Okamoto, Y.; Gong, X.; Kuroda, C.; Tori, M. *Tetrahedron* **2013**, *69*, 8505–8510.
9. Tori, M.; Tanio, Y.; Okamoto, Y.; Saito, Y.; Gong, X.; Kuroda, C.; Hanai, R. *Heterocycles* **2008**, *75*, 2029–2034.
10. 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.
11. 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.
12. Saito, Y.; Sasaki, Y.; Ohsaki, A.; Okamoto, Y.; Gong, X.; Kuroda, C.; Tori, M. *Tetrahedron* **2014**, *70*, 9726–9730.
13. (a) Moscowitz, A.; Charney, E.; Weiss, U.; Ziffer, H. *J. Am. Chem. Soc.* **1961**, *83*, 4661–4663; (b) Djerassi, C.; Records, R.; Bunnennberg, E.; Mislow, K.; Moscowitz, A. *J. Am. Chem. Soc.* **1962**, *84*, 870–872.
14. Li, Y.-S.; Wang, Z.-T.; Zhang, M.; Zhou, H.; Chen, J.-J.; Luo, S.-D. *Planta Med.* **2004**, *70*, 239–243.
15. Wang, W.-S.; Gao, K.; Jia, Z.-J. *J. Chin. Chem. Soc.* **2004**, *51*, 417–422.