Tetrahedron 70 (2014) 5878–5883

Contents lists available at ScienceDirect

Tetrahedron

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

Structure and cytotoxic evaluation of five 12-oxygenated eremophilanes from *Ligularia lingiana*

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ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form 7 June 2014
Accepted 8 June 2014
Available online 19 June 2014

Keywords:
Asteraceae
Ligularia lingiana
Sesquiterpenoid
Eremophilane
Dimer

ABSTRACT

Five new 12-oxygenated eremophilane sesquiterpenoids bearing a B-ring enone system were isolated from *Ligularia lingiana* S.W. Liu (Asteraceae) collected in China. Compound **5** was a Diels–Alder adduct of 3α-angeloyloxy-7β*H*-8-oxoeremophila-9,11(13)-dien-12-al and the monoterpene, ocimene. Compound **1** showed cytotoxic activities against HeLa, HL60, Jurkat, and NIH-3T3 cells at IC_{50} 0.0407, 0.440, 0.313, and 3.48 μM, respectively.

1. Introduction

The Hengduan Mountain area of China is of great interest from the viewpoint of rich plant diversity. The intra-specific diversity of some *Ligularia* specimens, herbaceous perennial plants of the Asteraceae species, collected in this area has been established.[1–9] We proposed a hypothesis that the production of furanoeremophilanes is ecologically advantageous over that of eremophilan-8-one derivatives.[10] In the course of our continuing studies on *Ligularia*, a sample of *Ligularia lingiana* S.W. Liu was collected in the Sichuan Province of China located at an altitude of 3700 m. Five new compounds **1**–**5** and the known 3α-angeloyloxy-7β*H*-eremophila-9,11-dien-8-one (**6**)[11] were isolated. The major constituent of the extract of *L. lingiana* was 3α-angeloyloxy-7β*H*-8-oxoeremophila-9,11(13)-dien-12-al (**1**). Herein, we describe the isolation and structural elucidation of the chemical constituents of the EtOAc extracts from the roots of this species.

2. Results and discussion

The roots of *L. lingiana* were extracted with EtOAc to afford an organic residue, which was separated by column chromatography and HPLC to give five new compounds, **1**–**5** (Fig. 1), as well as the known sesquiterpenoid 3α-angeloyloxy-7β*H*-eremophila-9,11-dien-8-one (**6**).[11] The new compounds all have oxygen functionalities at C-12 position.

The molecular formula of compound **1** was determined as $C_{20}H_{26}O_4$ by HRMS and ^{13}C NMR data. The ^1H NMR spectrum of compound **1** showed the presence of a formyl proton (δ_H 9.34) and an angeloyloxy (δ_H 5.76, 1.99, 1.88) moiety (Table 1). The ^{13}C NMR spectrum showed the presence of four methyl, four methylene, six methine, and six quaternary carbons (Table 2). The COSY data showed H_2-1/H_2-2/H-3/H-4/H_3-15, H_2-6/H-7, and H-3′/H_3-4′ connectivities. The HMBC spectrum displayed correlations between H_3-14 and C-4, C-5, C-6, and C-10, between H_2-6 and C-8, between H-9 and C-1, and between H_2-13 and C-7, C-11, and C-12. These

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http://dx.doi.org/10.1016/j.tet.2014.06.030
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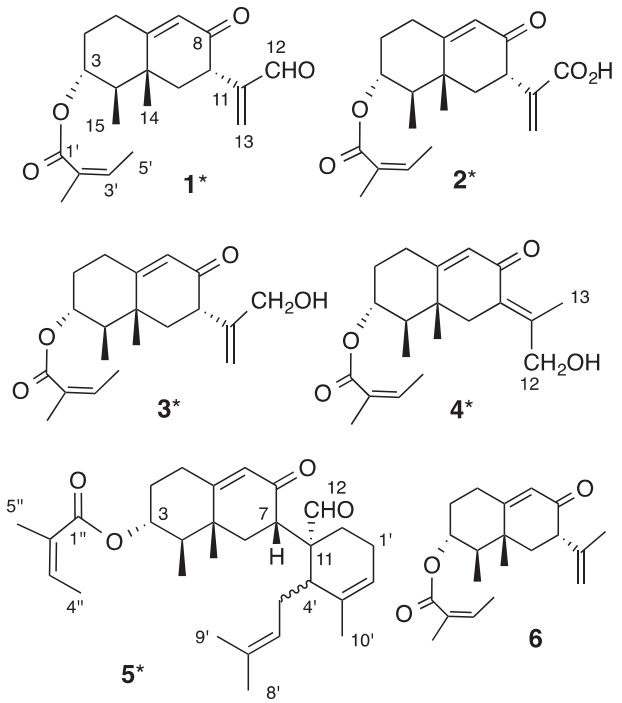

Fig. 1. Compounds isolated from *Ligularia lingiana* (*denotes new compound).

observations suggested the presence of an eremophilane skeleton for this compound, as shown in Fig. 2. Angeloyloxy substitution at C-3 was established through an HMBC correlation between H-3 and C-1′. The relative configuration was determined from NOESY correlations between H_3-14 and H-3β, between H_3-14 and H-7β, between H-6β and H_3-15, and between H-6α and H-4α. Therefore, the structure of compound **1** was established as 3α-angeloyloxy-7β*H*-8-oxoeremophila-9,11(13)-dien-12-al.

Compound **2** showed a tailing spot on TLC and its IR spectrum exhibited a broad absorption at 3520–3200 cm^{-1}; **2** was therefore assigned as a carboxylic acid. The molecular formula was determined as $C_{20}H_{26}O_5$ by HRMS and ^{13}C NMR data. The COSY, HMBC, and NOESY correlations of compound **2** were shown in Fig. 2 and were virtually the same as those for compound **1**. The carboxyl group at C-12 was determined by the HMBC correlation between H_2-13 and C-12 (δ_H 171.3). Therefore, the structure of compound **2** was established as 3α-angeloyloxy-7β*H*-8-oxoeremophila-9,11(13)-dien-12-oic acid.

Table 2
^{13}C NMR spectroscopic data (100 MHz, C_6D_6) of compounds **1**–**4**

Position	1	2	3	4
1	30.2	30.3	30.3	29.9
2	31.6	31.7	31.7	32.0
3	72.7	72.9	72.8	73.2
4	47.1	47.2	47.3	46.4
5	39.9	40.1	40.1	42.1
6	41.4	41.7	41.3	40.7
7	42.3	45.5	47.1	129.3
8	195.1	196.1	199.0	190.8
9	124.5	124.5	124.5	127.1
10	165.1	165.6	166.3	164.2
11	148.8	139.6	147.8	143.4
12	192.6	171.3	66.3	63.4
13	134.6	128[a]	113.6	18.1
14	16.4	16.6	16.3	17.0
15	10.3	10.5	10.4	10.8
1′	166.9	167.1	167.1	167.1
2′	128[a]	128[a]	128[a]	128[a]
3′	138.1	138.2	138.3	138.2
4′	15.8	16.0	16.0	15.9
5′	20.7	20.9	20.9	20.9

[a] The signal is superimposed on that of C_6H_6.

Compounds **3** and **4** showed the same molecular formula, $C_{20}H_{28}O_4$, indicating seven degrees of unsaturation. The ^{13}C NMR spectra of these compounds showed eight sp^2 carbons assignable to six olefinic carbons and two carbonyl groups (Tables 1 and 2), indicating that these molecules should be bicyclic. The COSY and HMBC spectra also suggested the presence of an eremophilane skeleton as shown in Fig. 1. Compound **3** had a double bond between C-11 and C-13, while compound **4** between C-7 and C-11. NOESY correlations indicated that the configurations at C-3, C-4, and C-5 were the same for each compound (Fig. 3). As evidenced by the NOE correlation between H-7 and H_3-14, the three-carbon unit at C-7 of compound **3** should be α-oriented. In the case of compound **4**, the geometry of the double bond between C-7 and C-11 was determined as *E*, as indicated by the observed NOE between H_2-12 and H-6β. Therefore, compounds **3** and **4** were established as 3α-angeloyloxy-7β*H*-12-hydroxyeremophila-9,11(13)-dien-8-one and 7(11)*E*-3α-angeloyloxy-12-hydroxyeremophila-7(11),9-dien-8-one, respectively.

Compound **5** exhibited a quasi-molecular ion peak at *m*/*z* 467, and its molecular formula was determined as $C_{30}H_{42}O_4$ by HRMS and ^{13}C NMR data. The ^1H NMR spectrum of **5** showed the presence

Table 1
^1H NMR spectroscopic data (400 MHz, C_6D_6) of compounds **1**–**4**

Position	δ_H (*J* in Hz)			
	1	2	3	4
1	1.66–1.74, m	1.63–1.72, m	1.60–1.70, m	1.69, ddd (14.8, 4.8, 2.7)
	1.87, tdd (14.3, 5.3, 1.8)	1.82–1.90, m	1.82, tdd (14.7, 5.3, 1.8)	1.79–1.91, m
2	1.13, dtd (14.3, 11.4, 4.5)	1.05–1.15, m	1.12, dtd (14.7, 11.4, 4.5)	1.16, dtd (14.1, 11.2, 4.5)
	1.93–2.02, m	1.89–1.98, m	1.91–1.98, m	1.94–2.01, m
3	4.89, td (11.4, 4.3)	4.90, td (11.2, 4.1)	4.89, td (11.4, 4.3)	4.92, td (11.2, 4.3)
4	1.28, dq (11.4, 6.8)	1.27, dq (11.2, 6.8)	1.23, dq (11.4, 6.7)	1.38, dq (11.2, 6.7)
6	1.66–1.74, m	1.76–1.86, m	1.60–1.70, m	1.82–1.89, m
	1.59, dd (12.8, 4.6)	1.63–1.72, m	1.60–1.70, m	2.68, d (13.7)
7	3.44, dd (14.2, 4.6)	3.38, dd (14.2, 4.3)	2.97, t (9.4)	—
9	5.65, d (1.8)	5.65, d (1.5)	5.59, d (1.8)	5.76, d (1.6)
12	9.34, s	—	4.23, d (12.8)	3.80, d (12.3)
	—	—	4.17, d (12.8)	3.75, d (12.3)
13	5.80, s	6.45, br d (0.8)	5.23, br d (1.2)	2.25, d (2.2)
	5.59, s	5.33, br s	4.84, br s	—
14	0.67, s	0.64, s	0.57, s	0.66, s
15	0.66, d (6.8)	0.68, d (6.8)	0.66, d (6.7)	0.79, d (6.7)
3′	5.76, qq (7.2, 1.5)	5.76, qq (7.2, 1.5)	5.75, qq (7.3, 1.5)	5.74, qq (7.2, 1.5)
4′	1.99, dq (7.2, 1.5)	2.00, dq (7.2, 1.5)	2.00, dq (7.3, 1.5)	2.00, dq (7.2, 1.5)
5′	1.88, quint (1.5)	1.89, quint (1.5)	1.89, quint (1.5)	1.88, quint (1.5)

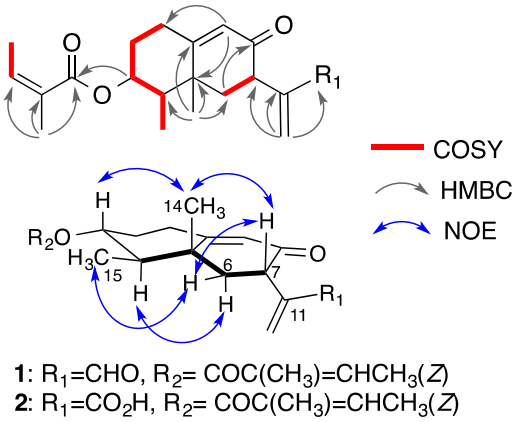

Fig. 2. Selected COSY, HMBC, and NOE correlations for compounds **1** and **2**.

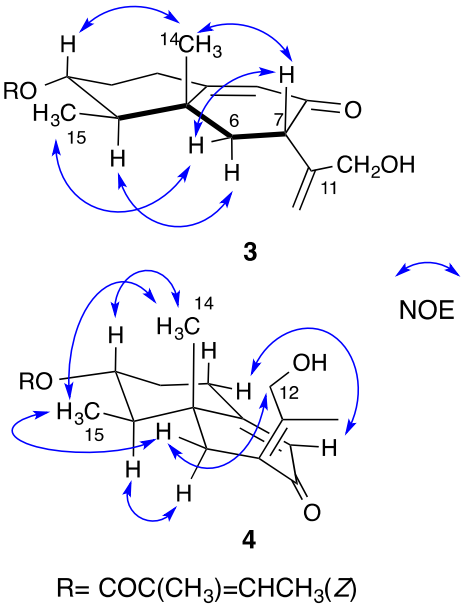

Fig. 3. Selected NOE correlations for compounds **3** and **4**.

of five methyl groups attached to sp^2 carbon atoms, and two methyl groups attached to sp^3 carbon atoms showing singlet and doublet splitting patterns. Four olefinic signals and a formyl proton (δ_H 10.08) were also detected. From these signals, the presence of an angeloyloxy group was suggested. The HMBC spectrum exhibited correlations between H_3-14 and C-4, C-5, C-6, and C-10, between H-9 and C-1, C-5, and C-7, and between H-12 and C-7, C-11, and C-13, suggesting that the left half of the molecule had an eremophilane skeleton with the formyl group at C-11. HMBC correlations between H_3-10′ and C-2′, C-3′, and C-4′, between H-4′ and C-11 and C-13, and between H_3-8′ and C-6′, C-7′, and C-9′ suggested that the right half of the molecule was derived from the monoterpene ocimene. An eremophilane skeleton connected with ocimene was proposed from these observations as shown in Fig. 4. The angeloyloxy group was assigned at C-3 from the HMBC correlation observed between H-3 and C-1″. These observations indicated that compound **5** was the Diels–Alder adduct of 3α-angeloyloxy-8-oxo-7β*H*-eremophila-9,11(13)-dien-12-al (**1**) and ocimene. Although many dimeric terpenoids have been isolated and identified,[12] a Diels–Alder-type compound derived from the monoterpene ocimene has not been reported to date.

Relative stereochemical assignments were deduced from NOESY experiments. Correlations between H-7β and H_3-14, between H_3-14 and H-3β, between H_3-15 and H-6β, and between H-6α and H-4α were observed (Fig. 4). The angeloyloxy moiety adopted a 3α-equatorial orientation, which was also supported by the coupling constants of H-3 (δ_H 4.92), which appeared as a triplet of doublets (J=12.2, 4.4 Hz). The configurations at C-11 and C-4′ could not be unambiguously determined because of the absence of decisive NOEs, although the NOE between H-4′ and H-6β was detected. The possible stereochemistry of Diels–Alder adduct is either **5a**, **5b**, **5c**, or **5d** (Fig. 4), **5a** and **5b** being *endo*-adducts and **5c** and **5d** *exo*-adducts. The stable conformations of **5a**–**5d** were calculated by CONFLEX.[13] In the case of **5a**–**5c**, H-4′ is close to H-6β, while in the case of **5d**, H-4′ is on the opposite side of C-6 (see Supplementary data). Because H_2-13 resonated at δ_H 2.61 and 2.15–2.09, deshielded probably due to its proximity to the C-8 carbonyl group, **5b** is excluded. This phenomenon is well explained by the conformation of either **5a** or **5c** as shown in Fig. 4. Therefore, this compound is formulated as either **5a** or **5c**.

The absolute configurations of compounds **1**–**3** were determined from the circular dichroism (CD) spectra to be those shown in Fig. 1. The compounds showed a negative Cotton effect around 325 nm (see Experimental), which is similar to that of known compound **6**.[11] The CD spectrum of compound **5** was nearly identical as those of compounds **1**–**3** and the absolute configuration was also established as depicted in Fig. 1. Compound **4** exhibited a split pattern in its CD spectrum, which is attributed to two α,β-unsaturated carbonyl moieties present in the molecule, one being cross-conjugated. Therefore, the absolute configuration of **4** was also established as shown.

Biological activities of compounds **1**–**5** were tested using HeLa, HL60, Jurkat, and NIH-3T3 cells. As shown in Table 3, among compounds isolated from this plant, compound **1** showed high activity against HeLa, HL60, and Jurkat cells. Compound **1** has a formyl group, specifically an α,β-unsaturated aldehyde moiety, which presumably acts as a Michael acceptor in the system, because compound **3**, bearing an allylic alcohol at this position, is 10–400 times less active. Compounds **2**–**5** showed very weak activities. It is noteworthy that, while compound **1** showed cytotoxicity against these cancer cell lines, it displayed considerable selectivity for HeLa cell lines; compound **1** was 85 times less active for NIH-3T3 cells than for HeLa cells, and 11 times less active than for Jurkat cells.

Eremophilanes bearing 3-acyloxy or hydroxy groups have been isolated from *Ligularia tongolensis*,[6] *Ligularia atroviolacea*,[6] *Ligularia pleurocaulis*,[7] *Ligularia dictyoneura*,[8] and *Ligularia franchetiana*,[9] as well as *Farfugium*,[14] *Petasites*,[15] and other species. We have isolated primarily 3-deoxy derivatives from *Ligularia virgaurea*,[1] *Ligularia kanaitzensis*,[2] *Ligularia vellerea*,[3] *Ligularia subspicata*,[4] and *Ligularia cyathiceps*.[5] *L. lingiana* can be further classified in a group that produces 3-acyloxyeremophilanes. The deacylated derivatives of **3** and **1** are known, the former being a natural product and the latter an oxidation product,[16] whose activities against the cell lines studied in this work have not been described. The 1α-acyloxy derivative of compound **1**, xylarenal A, is also known.[17] Some of its congeners have been recently isolated.[18] The isolation of a 3-deacyloxy derivative of compound **1** from *Cremanthodium stenactinium*, close species to *Ligularia*, was reported.[19] Compound **4** is a 3α-angeloyloxy derivative of kanaitzensol, previously isolated from *L. kanaitzensis*.[2b] Although some eremophilane sesquiterpenoids have been tested against some cancer cell lines,[20] they seldom show strong activities.[21]

3. Conclusion

In conclusion we have isolated six eremophil-9-en-8-one derivatives (five of which are as of yet unreported) from *L. lingiana* collected in the Sichuan Province of China. Compound **5** was the Diels–Alder adduct of **1** and the monoterpene, ocimene. The five

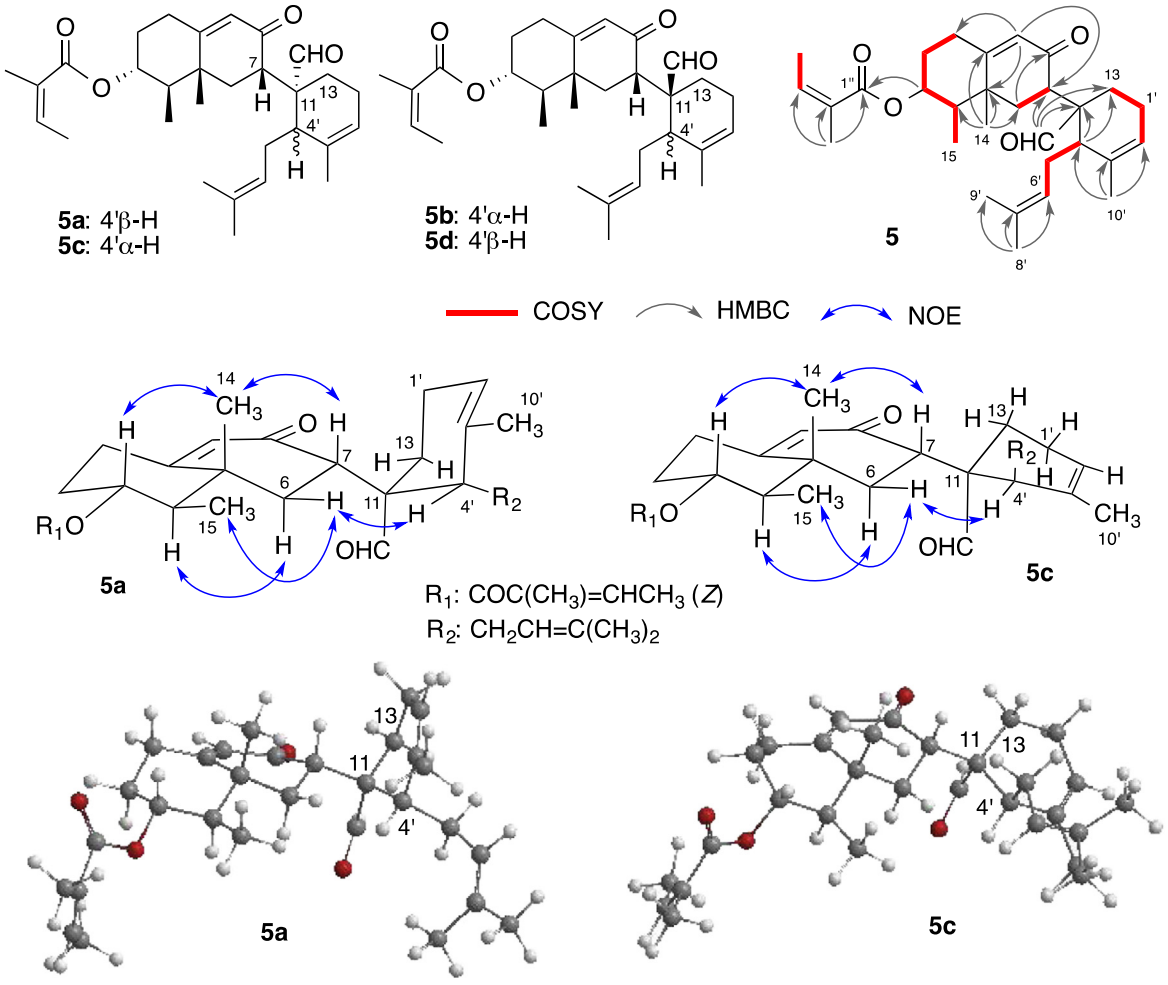

Fig. 4. Possible four Diels–Alder adducts, **5a**–**5d**, selected COSY, HMBC, and NOE correlations and conformations for compounds **5a** and **5c**.

Table 3
Biological activities of compounds **1**–**5** against HeLa, HL60, Jurkat, and NIH-3T3 cells (IC_{50}, μM)

	1	2	3	4	5	MMC[a]
HeLa	0.0407	27.8	16.2	3.20	28.5	0.339
HL60	0.440	4.26	4.34	3.52	4.29	0.0318
Jurkat	0.313	23.0	4.17	3.13	13.5	0.214
NIH-3T3	3.48	31.8	24.8	32.2	27.6	>0.598

[a] Mitomycin C was used as a positive control.

new compounds, bearing 12-oxygenated eremophilan-8-one structure, are biosynthetically related: these compounds must be precursors to furanoeremophilanes,[22] although compound **4** cannot cyclize to a furan. The most abundant compound was **1**, comprising 39.9% of the crude extract.

Compound **1** showed strong activity against HeLa, HL60, and Jurkat cells, but not against the NIH-3T3 cell line, meaning that the selectivity of the effectiveness is high. To the best of our knowledge, this is the first report about the chemical study on *L. lingiana*.

4. Experimental

4.1. General

Specific rotations and CD spectra were measured on a JASCO P-1030 and a 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 JEOL ECP 400 (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

L. lingiana S.W. Liu, collected in Gaoersishan, Sichuan (3700 m) in August 2007, was identified by G.X., one of the authors, and the voucher specimen, No. 2007-074, was deposited in the Herbarium of Kunming Institute of Botany.

4.3. Extraction and purification for structure determination

Roots of a sample (dry weight 3.15 g) were cut into pieces and were extracted with EtOAc to give 1.3 g of organic material. A part of this extract (744.9 mg) was separated using silica gel column chromatography (*n*-hexane/EtOAc, gradient) to give five fractions (A–E). Fraction A (59.2 mg) was further separated using HPLC (Nucleosil 50-5, *n*-hexane/EtOAc=9:1) to isolate **5** (5.0 mg) and **6** (11.0 mg). Fraction B (38.6 mg) was separated using HPLC (Nucleosil 50-5, *n*-hexane/EtOAc=4:1) to isolate **6** (4.9 mg). Fraction C was

compound **1** (290.8 mg). Fraction D (20.1 mg) also afforded compound **1** (6.4 mg) by HPLC (Nucleosil 50-5, *n*-hexane/EtOAc=7:3). Fraction E (72.3 mg) was separated using HPLC (Nucleosil 50-5, *n*-hexane/EtOAc=3:2) to isolate **2** (19.4 mg), **3** (7.9 mg), and **4** (3.2 mg).

4.4. Spectral data of the new compounds

4.4.1. Compound ***1***. Colorless oil; $[\alpha]_D^{23}$ +25 (*c* 0.9, EtOH); FTIR ν_{max} 1691, 1674 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 331 $[M+H]^+$, 259, 231 (base), 175, 148; CIHRMS *m*/*z* 331.1918 $[M+H]^+$ (calcd for $C_{20}H_{27}O_4$, 331.1910); CD: θ (EtOH) (λ_{max} nm) +1700 (215), +1300 (241), −1900 (328).

4.4.2. Compound ***2***. Colorless oil; $[\alpha]_D^{20}$ +34 (*c* 1.0, EtOH); FTIR ν_{max} 3520–3200, 1710, 1670 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; CIMS 347 $[M+H]^+$, 329, 247, 229 (base), 201; HRCIMS *m*/*z* 347.1853 $[M+H]^+$ (calcd for $C_{20}H_{27}O_5$, 347.1848); CD: θ (EtOH) (λ_{max} nm) +25,000 (220), +19,000 (241).

4.4.3. Compound ***3***. Colorless oil; $[\alpha]_D^{20}$ +40 (*c* 0.8, EtOH); FTIR ν_{max} 3400, 1709 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 333 $[M+H]^+$, 314 (base), 233, 215, 199, 159; HRCIMS *m*/*z* 333.2070 $[M+H]^+$ (calcd for $C_{20}H_{29}O_4$, 333.2066); CD: θ (EtOH) (λ_{max} nm) +52,985 (220), +43,984 (240), −1324 (325).

4.4.4. Compound ***4***. Colorless oil; $[\alpha]_D^{21}$ +32 (*c* 0.1, EtOH); FTIR ν_{max} 3400, 1715, 1655 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; CIMS *m*/*z* 333 $[M+H]^+$, 332 (base), 232, 215, 177; HRCIMS *m*/*z* 333.2054 $[M+H]^+$ (calcd for $C_{20}H_{29}O_4$, 333.2066); CD: θ (EtOH) (λ_{max} nm) +19,000 (251), −16,000 (279).

4.4.5. Compound ***5***. Colorless oil; $[\alpha]_D^{19}$ +93 (*c* 0.5, EtOH); FTIR ν_{max} 1713, 1670 cm^{-1}; ^1H NMR (400 MHz, C_6D_6): δ (ppm) 10.08 (1H, d, *J*=1.1 Hz, H-12), 5.76 (1H, qq, *J*=7.2, 1.5 Hz, H-3″), 5.56 (1H, d, *J*=1.6 Hz, H-9), 5.19 (1H, m, H-2′), 5.16 (1H, br t, *J*=7.3 Hz, H-6′), 4.92 (1H, td, *J*=11.4, 4.3 Hz, H-3β), 2.71 (1H, dd, *J*=14.4, 3.2 Hz, H-7β), 2.61 (1H, dd, *J*=13.9, 8.3 Hz, H-13), 2.38 (1H, t, *J*=5.7 Hz, H-4′), 2.20–2.12 (2H, m, H-6α, 5′), 2.15–2.09 (2H, m, H-13, 5′), 2.00 (3H, dq, *J*=7.2, 1.5, H-4″), 2.00–1.92 (1H, m, H-1′), 1.98–1.90 (2H, m, H-2β, 6β), 1.89 (3H, quint, *J*=1.5 Hz, H-5″), 1.89–1.80 (1H, m, H-1′), 1.67 (3H, q, *J*=1.6 Hz, H-10′), 1.62 (1H, m, H-1α), 1.60 (3H, br s, H-8′), 1.49 (3H, br s, H-9′), 1.32 (1H, dq, *J*=11.4, 6.8 Hz, H-4α), 1.08 (1H, tdt, *J*=14.4, 11.4, 4.5 Hz, H-2α), 0.79 (3H, d, *J*=6.8 Hz, H-15), 0.72 (3H, s, H-14); ^{13}C NMR (100 MHz, C_6D_6): δ (ppm) 209.2 (C-12), 199.4 (C-8), 167.1 (C-1″), 164.6 (C-10), 138.3 (C-3″), 136.3 (C-3′), 132.1 (C-7′), 127.9 (C-2″), 125.1 (C-9), 124.7 (C-6′), 121.9 (C-2′), 72.9 (C-3), 50.1 (C-11), 47.3 (C-4), 47.3 (C-7), 44.5 (C-4′), 40.6 (C-5), 38.6 (C-6), 31.6 (C-2), 31.6 (C-5′), 30.0 (C-1), 25.8 (C-8′), 23.9 (C-10′), 22.2 (C-13), 22.1 (C-1′), 20.9 (C-5″), 17.8 (C-9′), 16.0 (C-4″), 15.9 (C-14), 10.3 (C-15); CIMS *m*/*z* 467 $[M+H]^+$ (base), 449, 348, 331, 191, 176, 121; HRCIMS *m*/*z* 467.3163 $[M+H]^+$ (calcd for $C_{30}H_{43}O_4$, 467.3162); CD: θ (EtOH) (λ_{max} nm) +56,000 (204), +34,000 (239), −2200 (331).

4.4.6. Compound ***6***.[23] Colorless oil; $[\alpha]_D^{23}$ +45.1 (*c* 3.1, EtOH); FTIR ν_{max} 1720, 1680, 1640 cm^{-1}; ^1H NMR (500 MHz, C_6D_6): δ (ppm) 5.75 (1H, qq, *J*=7.3, 1.5 Hz, H-3′), 5.64 (1H, d, *J*=1.8 Hz, H-9), 5.01 (1H, quint, *J*=1.5 Hz, H-12), 4.91 (1H, td, *J*=11.0, 4.3 Hz, H-3), 4.82 (1H, br s, H-12), 2.88 (1H, dd, *J*=14.3, 4.5 Hz, H-7β), 2.00 (3H, dquint, *J*=7.3, 1.5 Hz, H-4′), 1.96 (1H, m, H-2β), 1.89 (3H, quint, *J*=1.5 Hz, H-5′), 1.87 (1H, m, H-1β), 1.84 (3H, s, H-13), 1.69 (1H, m, H-6α), 1.68 (1H, m, H-1α), 1.60 (1H, dd, *J*=14.0, 13.5 Hz, H-6β), 1.24 (1H, dq, *J*=11.0, 6.7 Hz, H-4), 1.16 (1H, m, H-2α), 0.67 (3H, d, *J*=6.7 Hz, H-15), 0.63 (3H, s, H-14); ^1H NMR (125 MHz, C_6D_6): δ (ppm) 196.6 (C-8), 167.0 (C-1′), 164.7 (C-10), 144.2 (C-11), 138.2 (C-3′), 128.5 (C-2′), 125.0 (C-9), 113.7 (C-12), 72.9 (C-3), 50.4 (C-7), 47.4 (C-4), 41.8 (C-6), 39.8 (C-5), 31.8 (C-2), 30.2 (C-1), 20.8 (C-5′), 20.6 (C-13), 16.5 (C-14), 15.9 (C-4′), 10.4 (C-15); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 199.0 (C-8), 168.1 (C-1′), 167.6 (C-10), 143.4 (C-11), 138.1 (C-3′), 127.9 (C-2′), 123.7 (C-9), 113.9 (C-12), 73.4 (C-3), 50.3 (C-7), 43.4 (C-4), 40.0 (C-5), 37.6 (C-6), 33.7 (C-2), 30.6 (C-1), 20.7 (C-13), 21.1 (C-14), 20.6 (C-5′), 15.8 (C-4′), 11.7 (C-15); CIMS *m*/*z* 317 $[M+H]^+$, 316, 217 (base), 216, 148; CD: θ (EtOH) (λ_{max} nm) +19,800 (216), +13,800 (234), −1900 (323).

4.5. Biological activities

IC_{50} values of compounds **1**–**4** were evaluated using the Cell Counting Kit (CCK-8) method (Dojindo, Japan). HeLa, HL-60, Jurkat, and NIH-3T3 cell lines were obtained from RIKEN BioResource Center (Tokyo, Japan). Phosphate-buffered saline (PBS), minimum essential medium (MEM), and RPMI-1640 medium were purchased from Gibco. These cell lines were cultured in the medium supplemented with 10% FBS (v/v) (Nichirei Bioscience, Japan) and 1% penicillin–streptomycin. DMSO was purchased from Wako Ltd., Japan.

HeLa and NIH-3T3 cell lines were cultured using MEM in 96-well plates for 24 h (5×103 cells/90 μL) at 37 °C under 5% CO_2. After that cells were treated with the isolated compounds at concentrations of 0.01, 0.1, 1.0, 10, and 100 μM for 48 h. A CCK-8 solution (10 μL) was added into each well and incubated for another 2 h. HL-60 and Jurkat cell lines were cultured using RPMI-1640 in 96-well plates for 2 h (8×103 cells/90 μL) at 37 °C under 5% CO_2. Then, cells were treated with the effected compounds against HeLa cell line at 0.01, 0.1, 1.0, 10, and 100 μM for 48 h. A CCK-8 solution (10 μL) was added into each well and incubated for another 4 h. Absorbances were measured at 450 nm using a microplate reader (2030 Multilabel Reader, ARVO X5, Perkin Elmer).

Acknowledgements

We thank Prof. X. Hao and Mrs. G. Hu of Kunming Institute of Botany for research coordination. This work was partly supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (No. 21404009).

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2014.06.030. These data include MOL files and InChiKeys of the most important compounds described in this article.

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23. As one of the reviewers suggested that the spectroscopic data of compound **6** should be included in the paper, all the data were provided here.