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Sesquiterpenoid with new skeleton from *Chloranthus henryi*

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Abstract—Dayejijiol (**1**), a novel sesquiterpene with a new carbon skeleton, and a novel labdane-type diterpenoid (13*S*)-13-hydroxy-19-methoxy-5α*H*-8(17), 14-labdadien (**3**), together with three known compounds chloranthalactone A (**4**), shizukanolide (**5**), shizukolidol (**6**) were isolated from *Chloranthus henryi* Hemsl. Their structures were established by a combination of 1D and 2D NMR spectroscopic techniques. Compounds **1** and **5** exhibited anti-tumor activities against Hela and K562 human tumor cell lines.

Chloranthus henryi Hemsl., Chinese name 'dayejiji', belonging to the family Chloranthaceae, has long been used as folk medicine in China.[1] Eudesmane-type and lindenane-type sesquiterpenoids are main constituents of the genus *Chloranthus*,[2] however, diterpenoids have not been reported from this genus. During the investigation for biologically active substances, we isolated[3] a new sesquiterpene with a novel bicarbocyclic framework, dayejijiol (**1**), and a new labdane-type diterpenoid (13*S*)-13-hydroxy-19-methoxy-5α*H*-8(17), 14-labdadien (**3**) together with two known lindenane-type sesquiterpenoids chloranthalactone A (**4**), shizukanolide (**5**) and one eudesmane-type sesquiterpenoid shizukolidol (**6**). Compounds **1** and **5** showed anti-tumor activities against Hela and K562 human tumor cell lines.

Compound **1** was isolated as a yellowish oil. The high resolution FT-ICR-MS exhibited an ion peak at *m*/*z* 287.1249 $[M+Na]^+$ (calcd, 287.1254), indicating the molecular formula as $C_{15}H_{20}O_4$. The IR spectrum revealed the presence of hydroxyl, α,β-unsaturated ester and exomethylene groups characterized by absorptions at ν_{max} 3441, 3085, 1757, 1640 and 893 cm^{-1}.

The ^1H NMR spectrum of **1** (Table 1) showed one methyl group at δ_H 0.92 (3H, d, $J = 6.5$, H-14) attached to the tertiary carbon and one methyl group appearing as a singlet at δ_H 1.93 (3H, s, H-12), attached to the quaternary carbon, and two typical exomethylene signals at δ_H 4.86, 5.04 (2H, 2s, H-15). The presence of two hydroxyl groups at δ_H 7.34 (1H, br s) and δ_H 3.83 (1H, br s) was revealed by a D_2O exchange experiment. Analysis of the ^{13}C NMR spectrum (Table 1) with the aids of the HMQC and DEPT experiments, revealed an ester carbonyl group at δ_C 172.82 (s, C-13), two double bonds at δ_C 143.30 (s, C-9), 114.66 (t, C-15), 158.52 (s, C-7), 121.37 (s, C-11), two oxyquaternary carbons at δ_C 73.46 (s, C-5), 104.78 (s, C-6), two methyl carbons at δ_C 10.67 (q, C-12), 23.11 (q, C-14), five methylene carbons at δ_C 34.47 (t, C-1), 44.73 (t, C-3), 27.37 (t, C-4), 48.27 (t, C-8), 114.66 (t, C-15), and two methine carbons at δ_C 49.91 (d, C-10), 27.24 (d, C-2). The carbon signals at δ_C 104.78 (s, C-6) suggested the presence of a dioxyquaternary carbon.[4] Since three out of six degrees of unsaturation from the molecular formula $C_{15}H_{20}O_4$ were accounted for, compound **1** was inferred to contain three rings.

Analysis of HMBC data led to three substructures. Correlations from H-2α (δ_H 1.87) to C-1 (δ_C 34.47), C-10 (δ_C 49.91), C-3 (δ_C 44.73), C-4 (δ_C 24.37), and from the methyl signals at Me-14 (δ_H 0.92, d, $J = 6.5$ Hz) to C-1 (δ_C 34.47), C-2 (27.24) and C-3 (44.73), indicated the presence of a structural unit ring A with the Me-14 attached to C-2. Ring B moiety was formed by connecting the structural unit COO–CO–CH and the unit COO–C–CH_2–C=CH_2 through an exomethylene group at C-9 (δ_C 143.30), which was judged by the HMBC correlations from H-10β (δ_H 2.04, 1H, d, $J = 14.0$) to C-9 (δ_C 143.30), C-15 (δ_C 114.66), C-5 (δ_C 73.46) and C-6 (δ_C 104.78), from H-15 (δ_H 5.05, 4.86, 2H, 2s) to C-10 (δ_C 49.91), C-8 (δ_C 48.27), from H-8α (δ_H 2.94, 1H, d, $J = 14.2$), H-8β (δ_H 2.33, 1H, d, $J = 14.2$) to C-7 (δ_C 158.52), C-6 (δ_C 104.78), C-11 (δ_C 121.37), C-15 (δ_C

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Table 1. NMR spectral data for compound **1** at 500 MHz NMR in DMSO-d_6

C/H No.	δ_H[a] mult. (J in Hz)	δ_C[b,c]	DEPT	^{1}H–^{1}H COSY	HMBC[d]
1α	0.99 (m)	34.47 (t)	CH_2	2, 10	2, 3, 5, 9, 10, 14
1β	1.71 (m)				
2α	1.87 (m)	27.24 (d)	CH	1, 3, 14	1, 3, 4, 10, 14
3α	1.53 (m)	44.73 (t)	CH_2	2, 4	1, 2, 5, 14
3β	2.16 (m)				
4α	1.73 (m)	24.37 (t)	CH_2	3	2, 3, 5, 6, 10
4β	1.58 (m)				
5		73.46 (s)	C		
6		104.78 (s)	C		
7		158.52 (s)	C		
8α	2.33 (d, $J = 14.2$)	48.27 (t)	CH_2		6, 7, 9, 10, 11,15
8β	2.94 (d, $J = 14.2$)				
9		143.30 (s)	C		
10	2.04 (d, $J = 14.0$)	49.91 (d)	CH	1	1, 2, 4, 5, 6, 8, 9, 15
11		121.37 (s)	C		
12	1.93 (s)	10.67 (q)	CH_3		7, 11, 13
13		172.82 (s)	C		
14	0.92 (d, $J = 6.5$)	23.11 (q)	CH_3		1, 2, 3
15	4.86, 5.05 (2s)	114.66 (t)	CH_3		8, 9, 10

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] Multiplicities inferred from DEPT and HMQC experiments.
[d] Proton showing long-range correlation with indicated carbons.

114.66), and C-9 (δ_C 143.30). HMBC correlations from H-12 (δ_H 1.93, 3H, *s*) to C-11 (δ_C 121.37), C-13 (δ_C 172.82) and C-7 (δ_C 158.52) suggested that the ring C moiety was constructed by connecting the structural unit COO–C–C–CH_3 through a lactone ring at C-13 (δ_C 172.82).

Taking into account the six degrees of unsaturation, and correlations of ^{1}H–^{1}H COSY from H-1β (δ_H 1.71) and H-1α (δ_H 0.99) in ring A to H-10β (δ_H 2.08) in ring B, and HMBC correlations from H-1β (δ_H 1.71) and H-1α (δ_H 0.99) in ring A to C-9 (δ_C 143.30) in ring B, and from H-4β (δ_H 1.58) and H-4α (δ_H 1.73) in ring A to C-10 (δ_C 49.91), C-5 (δ_C 73.46) and C-6 (δ_C 104.78) in ring B, and from H-8β (δ_H 2.94) and H-8α (δ_H 2.88) in ring B to C-11 (δ_C 121.37), C-6 (δ_C 104.78) and C-7 (δ_C 158.52) in ring C (Table 1) enabled the above part structural units to be assembled to give a gross structure named dayejijiol. The carbon skeleton of **1** was different from cadinane (methyl groups at C-3, 9),[5] valeriane (methyl groups at C-5, 19),[6] eremophilane (methyl groups at C-4, 5),[7] and eudesmane (methyl groups at C-4, 10),[8] named as chloranthane after the genus *Chloranthus*.

The relative stereochemistry of **1** was determined through 2D NOESY analysis. The observation of the NOESY correlation from H-4β (δ_H 1.58) to Me-14 (δ_H 0.92) suggested that Me-14 was in the β-configuration. Similarly, a NOSEY correlation from H-10 (δ_H 2.04) to Me-14 (δ_H 0.92) and H-4β (δ_H 1.58) suggested that H-10 and Me-14 were on the β face of the ring. The relative stereochemistry of C-5 and C-6 were achieved by a methylation experiment.[9] The observation of NOESY correlations of **2** methylated from **1** showed cross peaks from OCH_3-5 (δ_H 3.37) to H-3α (δ_H 1.53) and H-1α (δ_H 0.99) revealed that OCH_3-5 was α-configuration. Cross peaks from OCH_3-6 (δ_H 3.42) to H-4α (δ_H 1.73) and H-8α (δ_H 2.33) suggested that OCH_3-6 was also α-configuration. From above, ring A and ring B in **1** were trans-fused, and the hydroxyl groups of **1** at C-5 and C-6 were also in an α-configuration as shown in Figure 1.

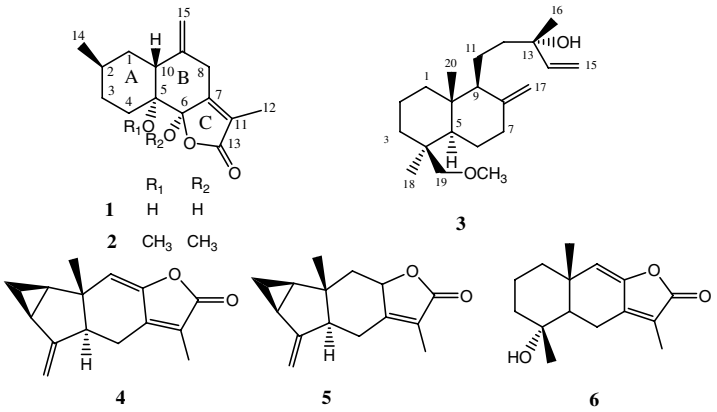

Figure 1. The structures of compounds **1**–**6**.

Compound **3** was isolated as a viscous, transparent oil. The IR spectrum showed peaks at 3435 cm^{-1} and 1645 cm^{-1}, suggesting the presence of a hydroxyl group and double bonds. The high resolution FT-ICR-MS exhibited an ion peak at *m/z* 321.2785 $[M+H]^+$ (calcd, 321.2788). The molecular formula was determined to be $C_{21}H_{36}O_2$ with the aids of ^1H and ^{13}C NMR data.

In the ^1H NMR spectrum (Table 2) of **3**, two signals at δ_H 4.46 and 4.76 (2H, 2s, H-17) were characteristic of the exomethylene moiety at C-8 found in labdane diterpenoids.[10] Two more terminal methylene at δ_H 4.94 and 5.12 (2H, 2dd $J = 17.4$, 10.8, H-15) were also a characteristic moiety at C-15 found in labdane diterpenoids.[10]

The ^{13}C NMR spectrum (Table 2) of **3** analyzed with the aid of the HMQC and DEPT spectra, revealed 21 carbon atoms including four methyl, ten methylene, three methine, and four quaternary carbons. The exomethylene at C-8 was characterized on this spectra by signals at δ_C 107.46 (t, C-17) and δ_C 149.28 (s, C-8). The terminal methylene at C-14 and C-15 was characterized by signals at δ_C 147.26 (s, C-14) and 111.82 (t, C-15). All the above data as well as those from the HMBC spectrum (Fig. 2) led to the completed structure elucidation of compound **3**.

The relative stereochemistry of **3** was achieved by analysis of its NOESY spectrum (Fig. 2). NOESY correlations from H-19 to H-2β and H-20, from H-20 to H-11, and from H-18 to H-3α and H-5, indicated that two rings were trans-fused, and the methoxyl groups at C-9 and C-19 were in α-orientations.

Table 2. NMR spectral data for compound **3** at 500 MHz NMR in DMSO-d_6

C/H No.	δ_H[a] mult. (*J* in Hz)	δ_C[b,c]
1α	0.82 (m)	39.69 (t)
1β	1.71 (m)	
2α	1.86 (m)	18.48 (t)
2β	1.54 (m)	
3α	0.85 (m)	36.19 (t)
3β	1.73 (m)	
4		39.12 (s)
5	1.28 (m)	56.60 (d)
6α	1.74 (m)	24.94 (t)
6β	1.23 (m)	
7α	1.80 (td, $J = 12.6$, 4.1)	39.01 (t)
7β	2.94 (d, $J = 11.8$)	
8		149.28 (s)
9	1.52 (m)	57.77 (d)
10		41.01 (s)
11	1.30, 1.55 (2m)	18.48 (t)
12	1.24, 1.52 (2m)	42.27 (t)
13		72.77 (s)
14	5.83 (dd, $J = 17.4$, 10.8)	147.26 (d)
15	4.94, 5.12 (2dd, $J = 17.4$, 10.8)	111.82 (t)
16	1.27 (s)	28.78 (q)
17	4.46, 4.76 (2s)	107.46 (t)
18	0.89 (s)	28.50 (q)
19	3.48, 3.14 (2d, $J = 10.3$)	63.38 (t)
20	0.57 (s)	16.10 (q)
OCH_3	3.38 (s)	53.78 (q)

[a] Recorded at 500 MHz.
[b] Recorded at 125 MHz.
[c] Multiplicities inferred from DEPT and HMQC experiments.

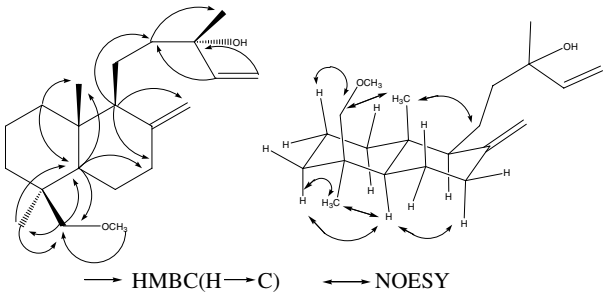

Figure 2. Key HMBC and NOESY correlations for compound **3**.

The ^{13}C NMR datum of C-13 (δ_C 72.77) of compound **3** was in close agreement with that of known similar 13(*S*) labdane-type diterpenoids in the literature,[11] which demonstrated that **3** possessed a 13(*S*)-configuration. On the basis of all spectroscopic evidences **3** was a novel labdane-type diterpenoid (13*S*)-13-hydroxy-19-methoxy-5α*H*-8(17), 14-labdadien.

Furthermore, two known lindenane-type sesquiterpenoids chloranthalactone A **4**, shizukanolide **5**, and one eudesmane-type sesquiterpenoid shizukolidol **6** were identified by comparison of their spectroscopic data with those of literature.[2,12]

Compounds **1**–**6** were tested for anti-tumor activity in vitro against two human tumor cell lines using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[13] DDP was used as positive control with IC_{50} value of 4.3 μg/mL against human cervical squamous carcinoma cell (Hela) and IC_{50} value of 3.5 μg/mL against human erythroleukemia (K562). Dayejijiol **1** showed strong anti-tumor activities against Hela with IC_{50} value of 5.6 μg/mL, and K562 with IC_{50} value of 5.0 μg/mL, respectively. Shizukanolide **5** showed moderate anti-tumor activities against Hela with IC_{50} value of 17.2 μg/mL, and K562 with IC_{50} value of 21.6 μg/mL, respectively. However compounds **2**–**4**, and **6** did not show anti-tumor activities ($IC_{50} > 100$ μg/mL).

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3. The shade-dried leaves and stems (3 Kg) were extracted with methanol, and 54 g extract was obtained, which was partitioned with petroleum ether, EtOAc, and BuOH successively. The petroleum ether extract (23 g) was subjected to column chromatography (CC) over silica gel (200–300 mesh, 800 g silica gel) eluting with petroleum ether/EtOAc (10:0–0:10, gradients) to afford 3 fractions. Fraction 1 was separated on silica gel CC (300–400 mesh, 100 g) repeatedly, using *n*-hexane/acetone (10:1) as the eluent to yield pure **3** (12.3 mg) and **4** (8.3 mg). Fraction 2 was rechromatographed on a silica gel (300–400 mesh, 60 g) column with *n*-hexane/acetone (5:1) to give pure **5** (10.8 mg). Fraction 3 was rechromatographed on a silica gel (300–400 mesh, 30 g) column with *n*-hexane/acetone (3:1) to give pure **6** (8.7 mg) and **1** (14.5 mg).
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9. Compound **1** (8 mg) was methylated by refluxing overnight with CH_3I–K_2CO_3 in acetone. The crude product was subjected to Prep-HPLC (Waters 600 HPLC, Shimpack Prep-ODS 250 × 20 mm, flow rate 8 mL/min, UV detector 210 nm), using CH_3OH/H_2O 75:25 as the eluent to afford compound **2** (3.6 mg, t_R 54.2 min) as a gum. ^1H NMR (acetone-d_6, 500 MHz): δ_H 4.93 (1H, s, H-15), 5.10 (H, s, H-15), 3.42 (3H, s, OCH_3-6), 3.37 (3H, s, OCH_3-5), 2.90 (1H, d, $J = 14.6$, H-8β), 2.36 (d, $J = 14.6$, H-8α), 2.14 (1H, m, H-3β), 2.07 (IH, d, $J = 14.3$, H-10), 1.91 (1H, s, H-12), 1.85 (1H, m, H-2α), 1.72 (1H, m, 4α), 1.70 (1H, m, H-1β), 1.61 (1H, m, H-4β), 1.52 (1H, m, H-3α), 1.02 (1H, m, H-1α), 0.88 (3H, d, $J = 6.7$, H-14).
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