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Senedensiscins A–F: six new eudesmane sesquiterpenoid glucosides from *Senecio densiserratus*

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ABSTRACT

Six new highly oxygenated eudesmane sesquiterpenoid glucosides, senedensiscins A–F (**1**–**6**), were isolated from the aerial parts of *Senecio densiserratus*. Their structures with the absolute configurations were established on the basis of spectroscopic analyses and chemical methods. These compounds represent an unprecedented type of sesquiterpenoid glucoside with the angeloyl group directly connected to the glucosyl moiety and their cytotoxicity was evaluated against the selected human cell lines, HL-60, SMMC-7721, and HeLa.

1. Introduction

The genus *Senecio* (Asteraceae) contains more than 1500 species widely distributed throughout the world and over 200 species of the genus are found mainly distributed in the northwest and southwest of China.[1] Most of these have long been used in Chinese folk medicine for their improvement of eyesight, detumescence, anti-inflammatory, vermifuge properties.[2] Pharmacological studies have shown that some *Senecio* species possess activities, such as antifatigue, anti-inflammation, anticancer, and immunomodulation effects.[3–7] Previously chemical investigations on this species have led to the isolation of various compounds belonging to eremophilane sesquiterpenes and pyrrolizidine alkaloids.[8–13] In our continuous efforts to find new anticancer and antimicrobial drugs or leads from Chinese herbal medicine,[14–17] an MeOH extract of the aerial parts of *Senecio densiserratus* exhibited a positive response to human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cell lines. Bioactivity-guided fractionation of the MeOH extract afforded six highly oxygenated eudesmane sesquiterpenoid glucosides (**1**–**6**) with modest cytotoxicity (Fig. 1). Their structures with the absolute configurations were established on the basis of spectroscopic analyses and chemical methods. A literature survey indicates that senedensiscins A–F (**1**–**6**) represent an unprecedented type of sesquiterpene glucoside with the angeloyl group directly connected to the glucosyl moiety. We present herein the details of isolation, structure determination, and cytotoxicity evaluation of these new products.

2. Results and discussion

2.1. Structure elucidation

Compound **1** was obtained as a colorless gum with a specific rotation of $[\alpha]_D^{20}$ −14 (*c* 0.3, CH_3OH). Its molecular formula was determined as $C_{28}H_{46}O_{10}$ by HRESIMS (*m*/*z* 565.2968 $[M+Na]^+$, calcd 565.2983) associated with NMR data (Tables 1 and 2), and indicated 5° of unsaturation. The IR spectrum showed absorption bands for hydroxyl groups (3449 cm^{-1}) and ester carbonyl groups (1739 cm^{-1}). An angeloyloxy group and an acetoxy group were present in **1**, as evidenced from the observed ^1H NMR signals [δ 6.18 (1H, qq, *J*=7.2, 1.6 Hz), 2.01 (3H, dq, *J*=7.2, 1.2 Hz), 1.92 (3H, dq, *J*=1.6, 1.2 Hz); δ 2.07 (3H, s)] in combination with the ^{13}C NMR signals [δ 169.5, 140.4, 126.9, 20.5, and 16.0; δ 171.0 and 20.7].[18,19] The NMR signals [δ_H 4.60 (1H, d, *J*=8.0 Hz) and δ_C 98.4, 78.2, 74.1, 72.6, 69.4, and 62.7] suggested that a β-glucopyranosyl unit was also present in **1**.[20] Besides these carbons of the above units, the ^{13}C NMR (DEPT) spectra of **1** also showed 15 carbon resonances due to 4 methyl, 4 methylene, 5 methine (2 oxygenated), and 2 quaternary carbons (1 oxygenated).

On the basis of these observations, **1** was considered to be a dicyclic sesquiterpene skeleton with an angeloyloxy unit, an

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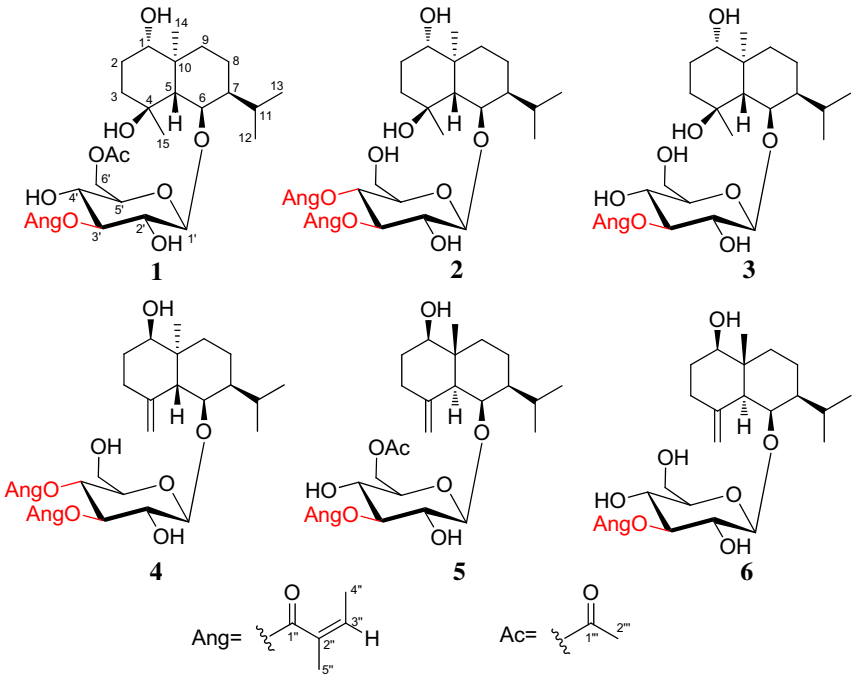


Fig. 1. Chemical structures of compounds **1**–**6**.

acetoxy group, a β-glucopyranosyl unit, and two hydroxyl groups. The $^{1}H-^{1}H$ COSY spectrum of **1** showed two main structural sequences, $-CH-CH_2-CH_2-$ and $-CH-CH-CH-[CH-(CH_3)_2]-CH_2-CH_2-$. The HMBC experiment showed correlations of H-1/C-5, C-9; H-6/C-4, C-10, C-11; H_3-12, H_3-13/C-7, C-11; H_3-15/C-3, C-4, C-5; and H_3-14/C-1, C-5, C-9, C-10 (Fig. 2). Consequently, **1** was determined as a highly oxygenated eudesmane sesquiterpenoid glucoside.

Table 1
^{1}H NMR Data of Compounds **1**–**6** in $CDCl_3$ (δ in ppm, J in Hz)

Position	**1**[b]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]
1	3.34, dd (7.2, 3.6)	3.37, dd (7.2, 3.6)	3.36, dd (7.2, 3.2)	3.46, dd (11.4, 4.8)	3.30, dd (11.6, 3.6)	3.31, dd (11.6, 3.6)
2α	1.60, m	1.58, m	1.57, m	1.65, m	1.71, m	1.72, m
2β	1.51, m	1.50, m	1.51, m	1.52, m	1.49, m	1.51, m
3α	1.69, m	1.70, m	1.71, m	1.92, m	2.25, m	2.27, m
3β	1.53, m	1.53, m	1.54, m	1.62, m	2.09, m	2.07, m
5	1.94, d (11.6)	1.98, d (11.6)	1.97, d (11.2)	2.28, d (10.4)	1.69, br s	1.70, br s
6	4.55, dd (11.6, 4.0)	4.54, dd (11.6, 4.4)	4.51, dd (11.2, 4.0)	4.37, dd (10.4, 4.2)	4.76, br s	4.73, br s
7	1.87, m	1.87, m	1.89, m	1.92, m	0.92, m	0.91, m
8α	1.79, m	1.78, m	1.79, m	1.78, m	1.70, m	1.72, m
8β	1.59, m	1.52, m	1.52, m	1.55, m	1.55, m	1.55, m
9α	1.51, m	1.53, m	1.51, m	1.62, m	2.01, m	2.02, m
9β	1.35, m	1.34, m	1.35, m	1.42, m	1.18, m	1.19, m
11	2.16, m	2.15, m	2.15, m	2.11, m	1.79, m	1.81, m
12	1.08, d (6.8)	1.09, d (6.6)	1.08, d (6.8)	1.04, d (6.6)	1.02, d (6.4)	1.01, d (6.4)
13	0.97, d (6.8)	0.98, d (6.6)	0.98, d (6.8)	0.98, d (6.6)	0.95, d (6.4)	0.95, d (6.4)
14	0.95, s	0.96, s	0.96, s	0.79, s	0.89, s	0.89, s
15	1.31, s	1.39, s	1.36, s	5.06, br s	5.53, br s	5.53, br s
				4.97, br s	4.82, br s	4.84, br s
1′	4.60, d (8.0)	4.70, d (7.8)	4.64, d (8.0)	4.66, d (7.8)	4.68, d (7.6)	4.73, d (7.6)
2′	3.57, dd (9.2, 8.0)	3.65, dd (9.6, 7.8)	3.57, dd (9.2, 8.0)	3.62, dd (9.6, 7.8)	3.52, dd (8.8, 7.6)	3.54, dd (9.2, 7.6)
3′	4.97, t (9.2)	5.36, t (9.6)	4.98, t (9.2)	5.39, t (9.6)	4.96, t (8.8)	4.96, t (9.2)
4′	3.60, m	5.16, t (9.6)	3.66, m	5.13, t (9.6)	3.60, m	3.72, m
5′	3.55, m	3.58, m	3.47, m	3.53, m	3.57, m	3.50, m
6′a	4.46, dd (12.0, 2.4)	3.79, dd (12.0, 2.4)	3.96, dd (12.0, 3.6)	3.77, br d (12.0)	4.67, br d (12.0)	3.93, dd (12.0, 3.6)
6′b	4.34, dd (12.0, 5.2)	3.64, dd (12.0, 4.8)	3.85, dd (12.0, 4.4)	3.63, dd (12.0, 4.8)	4.36, dd (12.0, 4.8)	3.86, dd (12.0, 4.2)
OAng						
3″	6.19, qq (7.2, 1.6)	6.12, qq (7.2, 1.6)	6.20, qq (7.2, 1.6)	6.11, qq (7.2, 1.6)	6.17, qq (7.2, 1.6)	6.19, qq (7.2, 1.6)
		6.09, qq (7.2, 1.6)		6.06, qq (7.2, 1.6)		
4″	2.01, dq (7.2, 1.2)	1.95, dq (7.2, 1.2)	2.02, dq (7.2, 1.2)	1.94, dq (7.2, 1.2)	2.01, dq (7.2, 1.2)	2.02, dq (7.2, 1.2)
		1.94, dq (7.2, 1.2)		1.93, dq (7.2, 1.2)		
5″	1.92, dq (1.6, 1.2)	1.82, dq (1.6, 1.2)	1.92, dq (1.6, 1.2)	1.83, dq (1.6, 1.2)	1.93, dq (1.6, 1.2)	1.93, dq (1.6, 1.2)
		1.79, dq (1.6, 1.2)		1.82, dq (1.6, 1.2)		
OAc						
2‴	2.07, s				2.07, s	

[a] Recorded at 600 MHz.
[b] Recorded at 400 MHz.

Table 2
^{13}C NMR data of compounds **1**–**6** in $CDCl_3$ (δ in ppm)

Position	1[b]	2[a]	3[a]	4[a]	5[b]	6[a]
1	79.5	79.6	79.8	79.4	80.6	80.5
2	28.0	28.0	28.0	30.9	31.1	31.2
3	39.3	39.4	39.4	35.5	35.1	35.1
4	72.3	72.1	72.2	145.4	145.4	146.1
5	49.7	49.9	49.9	47.9	52.0	52.0
6	78.9	79.5	79.5	74.5	71.0	71.5
7	41.7	41.7	41.7	39.7	53.3	53.3
8	23.2	23.1	23.1	22.7	20.5	20.5
9	35.2	35.2	35.2	31.9	38.1	38.1
10	41.4	41.6	41.7	42.1	40.5	40.6
11	25.3	25.4	25.4	25.6	29.5	29.3
12	22.6	22.6	22.5	22.3	20.6	20.7
13	23.2	23.5	23.5	23.2	22.3	22.3
14	13.7	13.8	13.9	12.4	13.9	13.9
15	23.1	23.0	22.9	110.3	109.9	109.5
1′	98.4	99.6	99.2	98.6	100.1	100.4
2′	72.6	73.4	72.8	73.6	74.2	75.7
3′	78.2	74.1	78.6	75.0	78.4	78.4
4′	69.4	68.1	69.8	68.5	69.6	70.1
5′	74.1	74.6	75.8	73.9	73.2	73.2
6′	62.7	61.5	62.4	61.5	62.9	62.6
OAng						
1″	169.5	167.7	169.6	167.2	169.5	169.6
		167.1		167.0		
2″	126.9	127.1	124.8	127.3	127.1	127.0
		126.7		126.7		
3″	140.4	140.4	140.6	140.2	140.1	140.3
		139.4		138.7		
4″	16.0	15.8	16.0	15.8	16.0	16.0
		15.8		15.7		
5″	20.5	20.4	20.5	20.4	20.5	20.5
		20.3		20.4		
OAc						
1‴	171.0				171.1	
2‴	20.7				20.7	

[a] Recorded at 150 MHz.
[b] Recorded at 100 MHz.

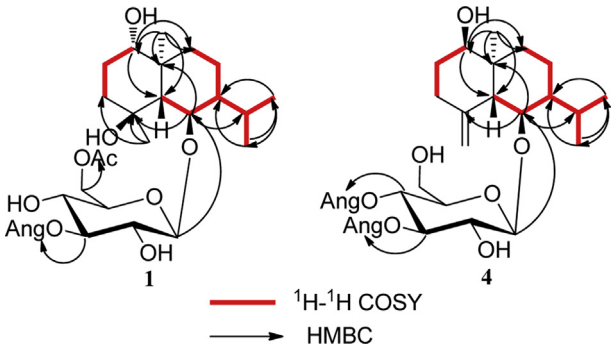
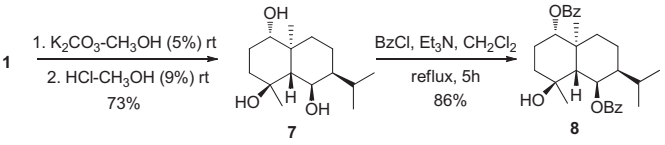


Fig. 2. Selected 2D NMR correlations for senedensiscin A (**1**) and D (**4**).

Furthermore, the β-glucopyranosyl was placed on C-6 due to the HMBC correlation from the anomeric proton at δ 4.60 to C-6. The cross-peaks of H-3′ and H-6′ with the ester carbonyl carbons of the angeloyloxy and acetoxy groups indicated their attachment to C-3′ and C-6′, respectively, (Fig.2). Therefore, the two hydroxyl groups must be located at C-1 and C-4, permitting the assignment of the molecular structure of **1**.

To confirm the structure of **1**, **1** was deacylated and then the product was hydrolyzed under acidic condition to yield **7** and glucose (Scheme 1).[21] The structure of the former was confirmed by comparison of its experimental and reported NMR data, while the sugar was identified as D-(+)-glucose by TLC comparison with an authentic sample and by its optical rotation value (Supplementary data).[20,22] Compound **7** was obtained as prisms and subjected to X-ray diffraction analysis (Fig. 3). Thus, the relative configuration of **1** was assigned from that of **7**.



Scheme 1. Hydroxylation and further benzoylation of compound **1**.

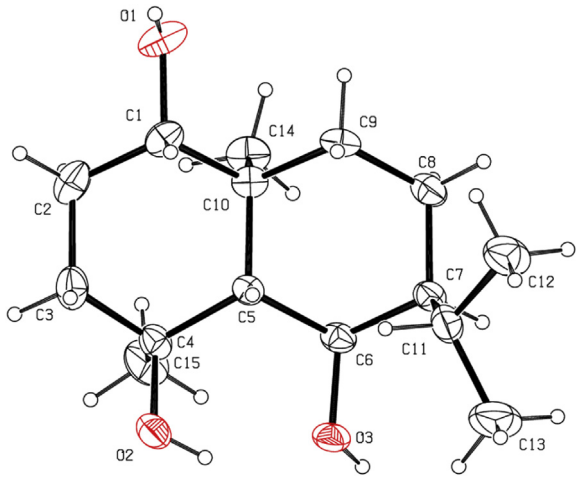


Fig. 3. ORTEP drawing of compound **7**.

The absolute configuration of **1** was based on that of **8**, which was, in turn, obtained via benzoylation of **7** and application of the electronic circular dichroism (ECD) exciton chirality method. The ECD spectrum of **8** showed the first negative Cotton effect at λ_{max}=248 nm corresponding to the exciton coupling between the benzoyl groups at C-1 and C-6. The counterclockwise manner of the two chromophores in space thus defined the absolute configuration of **8** as 1*S*,4*S*,5*R*,6*R*,7*S*,10*S* (Fig. 4).[23] Accordingly, the absolute configuration of **1** was determined. Compound **1** was apparently a rare enantio-eudesmane glucoside, which was named senedensiscin A.

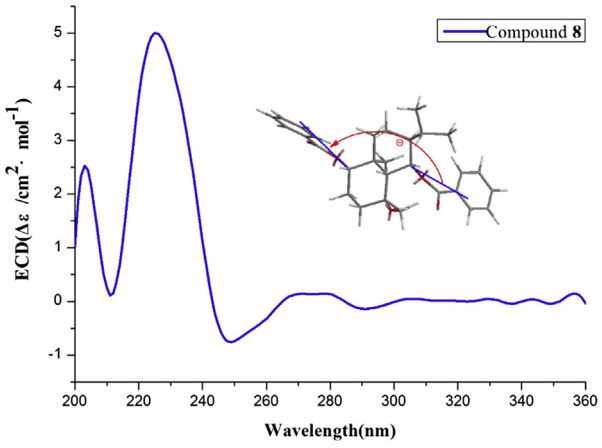


Fig. 4. CD spectra (in MeOH) of compounds **8**. Bold lines denote the electric transition dipole of the chromophores.

Compound **2** was obtained as a colorless gum and its HRESIMS (*m/z* 605.3289 $[M+Na]^+$, calcd 605.3296) revealed a molecular formula of $C_{31}H_{50}O_{10}$. The NMR data of **2** were similar to those of **1** except for the presence of one more angeloyloxy group and the absence of one acetoxy group. The HMBC data revealed correlations of H-3′ and H-4′ to the carbonyl carbons of the two angeloyloxy groups, indicating their attachment to C-3′ and C-4′, respectively. The configuration of **2** was elucidated by the NOESY spectrum and coupling constants (Fig. 5). The $^3J_{1,2}$ values (dd, J=7.2, 3.6 Hz) showed that H-1 was in the β-orientation. A NOESY correlation between H-1 and H-5 indicated that H-5 was in the β-orientation. The large coupling constant between H-5 and H-6 ($^3J_{5,6}$=11.6 Hz) indicated that H-6 was in the α-orientation. Moreover, the NOESY correlations between H-6 and H-7/CH_3-14/CH_3-15 confirmed that H-7, CH_3-14, and CH_3-15 were α-orientated (Fig. 5). The sequential deacylation and acidic hydrolysis of **2** also afforded **7** and D-(+)-glucose with $[\alpha]_D^{20}$ +56 (*c* 0.06, H_2O).[20,22] Thus, senedensiscin B was characterized as **2**.

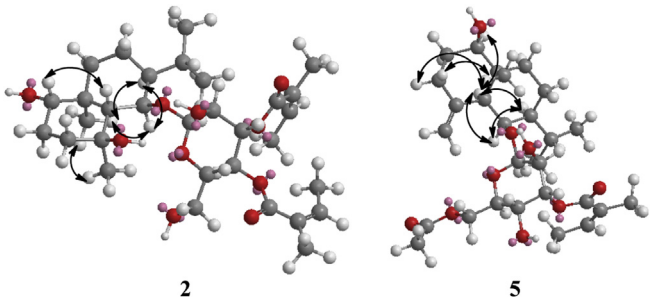


Fig. 5. Selected NOESY correlations (arrow) and relative stereochemistry for senedensiscin B (**2**) and E (**5**).

The molecular formula of senedensiscin C (**3**) was determined to be $C_{26}H_{44}O_9$ on the basis of the HRESIMS at *m/z* 523.2878 $[M+Na]^+$ (calcd for $C_{26}H_{44}O_9Na$, 523.2862) and ^{13}C NMR analyses, which indicated the absence of an angeloyloxy group compared to **2** (Tables 1 and 2). The HMBC correlation of H-3′ to the ester carbonyl carbon of the angeloyoxy group indicated its attachment to C-3′. Since the chemical conversions of **3** also afforded **7** and D-(+)-glucose by sequential deacylation and acidic hydrolysis (Supplementary data), compound **3** should share the same absolute configuration as **1** and **2**.

HRESIMS and ^{13}C NMR data for senedensiscin D (**4**) revealed the molecular formula of $C_{31}H_{48}O_9$. Based on a comparison of the NMR data of **4** (Tables 1 and 2) with those of **2**, the signals corresponding to one of the methyl groups in **2** had been replaced in **4** by those of an exocyclic olefinic bond [δ_H 4.97, 5.06 (each 1H, br s); δ_C 145.4 (C), 110.3 (CH_2)].[24] On the basis of the HMBC correlations of H-14a, 14b/C-3, C-4, and C-5, and H-2, H-6/C-4, the two carbons of the exocyclic double bond in **4** were assigned as C-4 and C-15. The HMBC correlations were also used to confirm that the positions of the β-glucopyranosyl moiety and the two ester groups of **4** were identical to those of **2** (Fig. 2). The $^3J_{1,2}$ values (dd, J=11.4, 4.8 Hz) showed that H-1 was in the α-orientation. Furthermore, the NOESY correlations of H-1/H_3-14, and of H_3-14/H-6, H-7 revealed that all of these protons were α-orientated. Finally, H-5 was determined to be in the β-orientation because of the large coupling constant between H-5 and H-6 ($^3J_{5,6}$=10.4 Hz). The absolute configuration of **4**, i.e., (1*R*,5*R*,6*R*,7*S*,10*S*), was determined via the application of the octant rule to the negative Cotton effect, which was observed at λ_{max}=206 nm as a consequence of the exocyclic double bond in **4** (Fig. 6).[25]

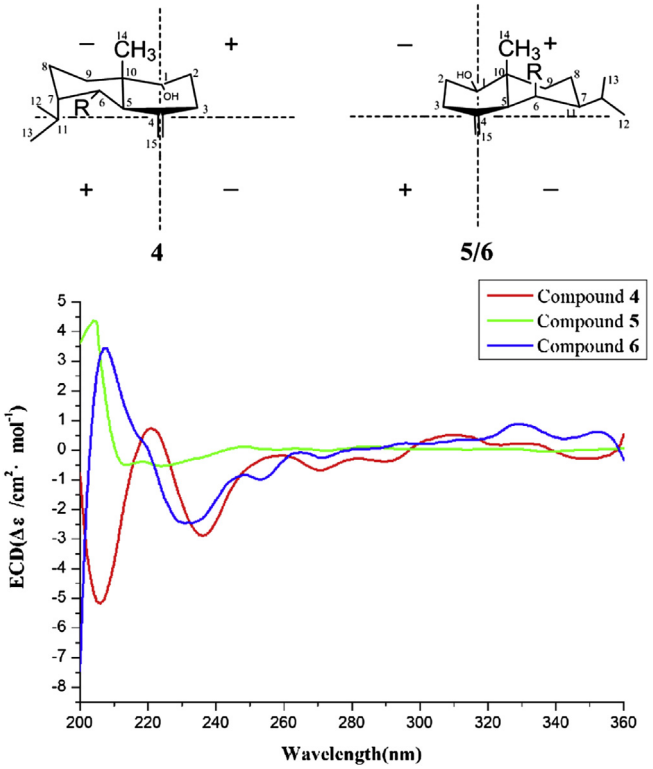


Fig. 6. CD spectra (in MeOH) of senedensiscin D (**4**), E (**5**), and F (**6**).

Compound **5** was obtained as a colorless gum and its molecular formula was determined as $C_{28}H_{44}O_9$ by HRESIMS at *m/z* 547.2878 $[M+Na]^+$ (calcd for $C_{28}H_{44}O_9Na$, 547.2878). The NMR spectra of **5** (Tables 1 and 2) were similar to those of **4** except for the presence of one acetoxy group and the absence of one angeloyloxy group, suggesting that **5** was also a highly oxygenated 6β-glucopyranosyl-1-hydroxyeudesm-4(15)-ene derivative. Key HMBC correlations of H-3′/C=O (OAng) and of H-6′a, H-6′b/C=O (OAc) indicated that the angeloyloxy and acetoxy groups were at C-3′ and C-6′, respectively.

The $^3J_{1,2}$ values (dd, J=11.6, 3.6 Hz) showed that H-1 was in the α-orientation. The NOESY correlations of H-1/H-5 and H-5/H-6, H-7 revealed that H-5, H-6, and H-7 were all α-orientated. The coupling constants of H-6/H-5 and H-6/H-7 were <1 Hz because their dihedral angles were about 90°, further confirming that the three protons were cofacial. Furthermore, the NOESY correlations of H-5/H-3α and H_3-14/H-3β revealed that H_3-14 was in the β-orientation (Fig. 5). Interestingly, the chemical shift of H-7 (δ 0.92) in **5** was lower than that in **4** (δ 1.92), and the difference was attributed to the different orientations of H_3-14 and H-5 in the two compounds. The absolute configuration of **5**, i.e., (1*R*,5*S*,6*R*,7*S*,10*R*), was determined via the application of the octant rule to the positive Cotton effect, which was observed at λ_{max}=204 nm as a consequence of the exocyclic double bond, (Fig. 6).[25] Thus, senedensiscin E was characterized as **5**.

The molecular formula of senedensiscin F (**6**) was determined as $C_{26}H_{42}O_8$ by the HRESIMS at *m/z* 505.2759 $[M+Na]^+$ (calcd for $C_{26}H_{42}O_8Na$, 505.2772). The NMR spectra of **6** showed similar signals to those of **5** except for the absence of an acetoxy group, and the upfield-shift of the proton signals of H-6′a and H-6′b (δ 3.93 and 3.86). These observations suggested that the key difference between compounds **5** and **6** was that the C-6′ *O*-acetyl moiety of **5** had been replaced with a hydroxyl group. Extensive analysis of $^1H-^1H$ COSY, HSQC, and HMBC data further confirmed this assignment. The specific rotation of **6** was determined to be −39 (*c* 0.3, MeOH), which was similar to the −57 (*c* 0.4, MeOH) rotation

observed for **5**. Furthermore, a positive Cotton effect was observed for **6** at λ_{max}=207 nm, which was similar to that of observed for **5** (Fig. 6). Taken together, these results indicated that compounds **5** and **6** had the same configurations.

2.2. Evaluation of cytotoxicity

The cytotoxicity of senedensiscins A–F (**1**–**6**) and compound **7** was evaluated against human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cell lines using the sulforhodamine B (SRB) method as previously reported.[26] The results are shown in Table 3. Interestingly, senedensiscins A–F, with the angeloyl group directly connected to the glucosyl moiety, showed moderate activity against the three lines ($IC_{50}\approx30$ μM) compared with compound **7** without these groups, which was inactive (IC_{50}>150 μM). Mitomycin was used as positive control with an IC_{50} value of 3 μM. These finding suggests that the angeloyloxy moieties connected with glucosyl units could be important in mediating the cytotoxicity of eudesmane sesquiterpenes. In addition, senedensiscins A–F, in which the angeloyloxy groups were connected to C-3′, or C-3′ and C-4′, showed no difference in their cytotoxicity against the three cell lines. This result suggests that the number and the location of the angeloyloxy groups might have no effects on the cytotoxicity of the compounds.

Table 3
IC_{50} values for cytotoxicity of compounds **1**–**6**

Compounds	IC_{50} (μM)		
	HL-60 cell	SMMC-7721 cell	HeLa cell
1	33.0±4.8	32.8±6.4	52.4±9.4
2	24.5±6.4	30.9±5.9	37.4±6.1
3	38.5±8.1	35.2±2.9	40.9±8.2
4	33.2±4.1	34.3±3.8	41.3±4.2
5	35.6±4.4	35.1±3.2	39.2±3.8
6	34.7±6.8	32.4±3.4	41.5±4.6
7	>150	>150	>150
Mitomycin	1.5±0.6	5.4±1.4	3.3±1.7

3. Conclusion

In summary, six new highly oxygenated eudesmane sesquiterpenoid glucosides, senedensiscins A–F, were isolated from the aerial parts of *S. densiserratus*. Their gross structures and relative stereochemistry were elucidated based on extensive NMR studies, and the absolute configurations of them were determined by chemical methods and CD analyses. To the best of our knowledge, these new ones are the first examples of sesquiterpene glucoside with the angeloyl group directly connected to the glucosyl moiety. Preliminary cytotoxicity studies against HL-60, SMMC-7721, and HeLa cell lines suggested that the angeloyloxy group in the sugar chain of these eudesmane sesquiterpene glycosides is critical for the cytotoxicity. These results might be the interesting topics for both natural products chemistry and pharmacology in the future.

4. Experimental section

4.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. IR spectra were recorded on a Nicolet NEXUS 670 FT-IR spectrometer. The ECD spectrum was obtained on a JASCO J-720 spectropolarimeter. NMR spectra were recorded on Varian Mercury-600BB NMR (600 MHz) and Bruker Avance III-400 (400 MHz) spectrometer with TMS as internal standard. HRESIMS data were measured on a Bruker Micro TOF-Q II spectrometer. The X-ray crystallographic data were collected on a Bruker Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. Silica gel (200–300 mesh) used for CC and silica gel GF_{254} (10–40 μm) used for TLC were supplied by Qingdao Marine Chemical Factory, Qingdao, PR China. Sephadex LH-20 used for CC was purchased from GE Healthcare Bio-Sciences AB, Uppsala, Sweden. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in EtOH (v/v). All chemicals used in the study were of analytical grade.

4.2. Plant material

The aerial parts of *S. densiserratus* were collected from Danchang County, Gansu Province, People's Republic of China, in August 2006, and authenticated by Prof. Guoliang Zhang from the School of Life Science, Lanzhou University. A voucher specimen (No. 20060826) was deposited at the Natural Product Laboratory of College of Chemistry and Chemical Engineering, Lanzhou University.

4.3. Extraction and isolation

The air-dried aerial parts of *S. densiserratus* (7.97 kg) were pulverized and extracted with MeOH (3×5 L, 7 days each) at room temperature. The solvent was evaporated under reduced pressure to obtain an extract (230 g), which was suspended in hot H_2O (60 °C, 0.5 L). This suspension was extracted successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction was concentrated under reduced pressure to afford a residue (96.5 g), which was subjected to silica gel column chromatography (200–300 mesh, 1200 g) with a gradient of petroleum ether–acetone (1:0, 30:1, 10:1, 5:1, 2:1, 1:1, 0:1) as eluent, and six fractions A–F were collected according to TLC analysis.

Fraction D (4.2 g) was separated on a silica gel column (200–300 mesh, 80 g) eluting with petroleum ether–EtOAc (8:1, 5:1, 2:1, 1:1) to give four fractions FrB.1–FrB.4. FrB.3 (1.5 g) was separated on a silica gel column (200–300 mesh, 30 g) using $CHCl_3$–EtOAc (15:1, 10:1, 5:1) to give three crude fractions FrB.3.1–FrB.3.3. FrB.3.2 (0.7 g) was further separated on a silica gel column (200–300 mesh, 10 g) with $CHCl_3$–EtOAc (10:1) as eluent to obtain **4** (2 mg) and **5** (12 mg). Fraction E (3.9 g) was separated on a silica gel column (200–300 mesh, 120 g) eluting with $CHCl_3$–EtOAc (10:1, 5:1, 2:1) to give four fractions FrC.1–FrC.4. FrC.2 (1.4 g) was separated on a silica gel column (200–300 mesh, 35 g) using $CHCl_3$–EtOAc (5:1, 2:1) to give four fractions FrC.4.1–FrC.4.4. FrC.4.2 (0.23 g) was further separated on a silica gel column eluting again with $CHCl_3$–EtOAc (5:1), followed by Sephadex LH-20 eluted with MeOH–$CHCl_3$ (1:1) to afford **3** (5 mg) and **6** (4 mg). FrC.4.3 (0.25 g) was further separated on a silica gel column eluting again with $CHCl_3$–EtOAc (5:1), followed by Sephadex LH-20 eluted with MeOH–$CHCl_3$ (1:1) to afford **1** (12 mg) and **2** (5 mg).

*4.3.1. Senedensiscin A (**1**).* Colorless gum; $[\alpha]_D^{20}$ −14 (*c* 0.3, CH_3OH); IR (KBr) ν_{max} 3449, 2924, 1739, 1643, 1461 cm^{-1}; ^1H NMR (400 Hz) and ^{13}C NMR (DEPT) (100 Hz) see Tables 1 and 2; HRESIMS *m*/*z* 565.2968 $[M+Na]^+$ (565.2983 calcd for $C_{28}H_{46}O_{10}Na$).

*4.3.2. Senedensiscin B (**2**).* Colorless oil; $[\alpha]_D^{20}$ −80 (*c* 0.1, CH_3OH); IR (KBr) ν_{max} 3443, 2928, 1726, 1646, 1461 cm^{-1}; ^1H NMR (600 Hz) and ^{13}C NMR (DEPT) (150 Hz) see Tables 1 and 2; HRESIMS *m*/*z* 605.3289 $[M+Na]^+$ (605.3296 calcd for $C_{31}H_{50}O_{10}Na$).

*4.3.3. Senedensiscin C (**3**).* Colorless oil; $[\alpha]_D^{20}$ −25 (*c* 0.3, CH_3OH); IR (KBr) ν_{max} 3434, 2919, 1714, 1645, 1464 cm^{-1}; ^1H NMR (600 Hz) and

^{13}C NMR (DEPT) (150 Hz) see Tables 1 and 2; HRESIMS *m*/*z* 523.2878 $[M+Na]^+$ (523.2862 calcd for $C_{26}H_{44}O_9Na$).

*4.3.4. Senedensiscin D (**4**).* Colorless oil; $[\alpha]_D^{20}$ −22 (*c* 0.1, CH_3OH); IR (KBr) ν_{max} 3440, 2923, 1725, 1648, 1462 cm^{-1}; ^{1}H NMR (600 Hz) and ^{13}C NMR (DEPT) (150 Hz) see Tables 1 and 2; HRESIMS *m*/*z* 587.3168 $[M+Na]^+$ (587.3191 calcd for $C_{31}H_{48}O_9Na$).

*4.3.5. Senedensiscin E (**5**).* Colorless oil; $[\alpha]_D^{20}$ −57 (*c* 0.4, CH_3OH); IR (KBr) ν_{max} 3439, 2935, 1716, 1648, 1456 cm^{-1}; ^{1}H NMR (400 Hz) and ^{13}C NMR (DEPT) (100 Hz) see Tables 1 and 2; HRESIMS *m*/*z* 547.2878 $[M+Na]^+$ (547.2878 calcd for $C_{28}H_{44}O_9Na$).

*4.3.6. Senedensiscin F (**6**).* Colorless oil; $[\alpha]_D^{20}$ −39 (*c* 0.3, CH_3OH); IR (KBr) ν_{max} 3443, 2932, 1723, 1648, 1456 cm^{-1}; ^{1}H NMR (600 Hz) and ^{13}C NMR (DEPT) (150 Hz) see Tables 1 and 2; HRESIMS *m*/*z* 505.2759 $[M+Na]^+$ (505.2772 calcd for $C_{26}H_{42}O_8Na$).

*4.3.7. Compound **8**.* Amorphous solid; $[\alpha]_D^{20}$ −45 (*c* 0.4, CH_3OH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.01 (4H, m, ArH), 7.58 (2H, m, ArH), 7.46 (4H, m, ArH), 5.75 (1H, dd, *J*=12.0, 4.0 Hz, H-6), 4.94 (1H, dd, *J*=7.2, 4.0 Hz, H-1), 2.35 (1H, d, *J*=12.0 Hz, H-5), 2.16 (1H, m, H-11), 1.92 (1H, m, H-7), 1.89 (1H, m, H-2a), 1.84 (1H, m, H-8a), 1.78 (1H, m, H-8b), 1.75 (1H, m, H-3a), 1.74 (1H, m, H-2b), 1.73 (1H, m, H-3b), 1.59 (1H, m, H-9a), 1.44 (1H, m, H-9b), 1.30 (1H, s, H-15), 1.22 (1H, s, H-14), 1.04 (1H, d, *J*=6.8 Hz, H-12), 0.92 (1H, d, *J*=6.8 Hz, H-13).

4.4. X-ray crystallographic analysis of 7

Single-crystal X-ray diffraction data for **7** were recorded on a computer-controlled Bruker Smart CCD diffractometer equipped with graphite-monochromated Mo Kα radiation (λ=0.71073 Å) at 296 (2) K. The structure was solved by Direct Method of SHELXS-97 and refined by full-matrix least-squares techniques using the SHELXL-97 program.[27] Crystallographic data for **7** have been deposited in the Cambridge Crystallographic Data Centre with the deposition number of CCDC 914837.

*4.4.1. Crystal data for **7**.* Colorless block crystal, $C_{15}H_{28}O_3$, *M*=256.37, orthorhombic, space group P_1; *a*=6.285 (4) Å, *b*=14.268 (10) Å, *c*=14.436 (10) Å, *V*=1125.6 (14) $Å^3$, *Z*=3, D_{calcd}=1.135 g/cm^3, λ=0.71073 Å, μ (MoKα)=0.077 mm^{-1}, and *F* (000)=426. The final indices were *R*=0.0486, R_w=0.1290 with goodness-of-fit=1.056.

4.5. Bioassays for cytotoxicity

The cytotoxicity of compounds **1**–**7** toward human leukemia (HL-60), human hepatoma (SMMC-7721), and human cervical carcinoma (HeLa) cell lines was determined in 96-well microtiter plates by the sulforhodamine B method. Briefly, the cells were harvested and seeded in 96-well plates with the final volume 100 μL containing 4×10^3 cells per well. After 24 h incubation, cells were treated with various concentrations of **1**–**7** for 48 h. The cultures were fixed at 4 °C for 1 h by addition of ice-cold 50% trichloroacetic acid to give a final concentration of 10%. Fixed cells were rinsed five times with deionized H_2O and stained for 10 min with 0.4% sulforhodamine B dissolved in 0.1% HOAc. The wells were washed five times with 0.1% HOAc and left to dry overnight. The absorbed sulforhodamine B was dissolved in 150 μL unbuffered 1% Tris base [tris-(hydroxymethyl) aminomethane] solution in H_2O (pH 10.5). The absorbance of extracted sulforhodamine B at 515 nm was measured on a microplate reader (Bio-Rad). The experiments were carried out in triplicate. Each run entailed five or six concentrations of the compounds being tested. The percentage survival rates of cells exposed to the compounds were calculated by assuming the survival rate of untreated cells to be 100%.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.10.047.

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