Sesquiterpene Lactones from *Scorzonera austriaca*

Ying Zhu,*[,†] Pei-Zhuo Hu,[†,‡] Zi-Wei He,[†] Quan-Xiang Wu,[†] Juan Li,[†] and Wang-Suo Wu[‡]

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, and Radiochemistry Laboratory, Lanzhou University, Lanzhou 730000, People's Republic of China

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Six new sesquiterpene lactones, scorzoaustriacoside (**1**), scorzoaustriacin (**2**), scorzoaustriacin 3-*O*-*β*-D-glucoside (**3**), 4-*epi*-dihydroestafiatol (**4**), 14-isovaleroxyscorzoaustricin (**5**), and 14-isovaleroxyscorzoaustricin sulfate (**6**), along with five known guaianolides, were isolated from an acetone extract of the roots of *Scorzonera austriaca*. The structures of the new compounds were elucidated mainly by interpretation of their 1D and 2D NMR and HRMS data. Several isolates obtained in this investigation were evaluated against a small panel of cancer cell lines.

The genus *Scorzonera* (Asteraceae) comprises more than 170 species distributed from the Mediterranean region to Central Asia. Among them, 23 species have been found in mountain areas at altitudes of 1000−3000 m in mainland China.[1] In Europe and Asia, the roots of certain *Scorzonera* species have been used as officinal plants due to their medicinal value.[2–5] *Scorzonera austriaca* Willd. has been employed as a Tibetan folk medicine for the treatment of fever, carbuncles, inflammation, and mastitis in the People's Republic of China.[5] Coumarins,[6] flavonoids,[6] and guaiane-type sesquiterpenoids[7,8] have been previously reported from this plant. We report herein the isolation and structure elucidation of six new sesquiterpenoids, namely, scorzoaustriacoside (**1**), scorzoaustriacin (**2**), scorzoaustriacin 3-*O*-*β*-D-glucoside (**3**), 4-*epi*-dihydroestafiatol (**4**), 14-isovaleroxyscorzoaustricin (**5**), and 14-isovaleroxyscorzoaustricin sulfate (**6**), along with five known guaianolides, from an acetone extract of the roots of *S. austriaca*. Scorzoaustriacin (**2**) is a guaianolide derivative possessing an unusual aminomethyl-*γ*-butyrolactone structural unit. Selected isolates obtained in this investigation were evaluated against a small panel of cancer cell lines.

A molecular formula of $C_{21}H_{32}O_9$ was assigned for compound **1** from the observed HRESIMS data at *m/z* 446.2376 ($[M + NH_4]^+$). The ^{13}C NMR and DEPT spectra of **1** showed 21 carbon signals including three methyls, three methylenes, 12 methines, and three quaternary carbons. The presence of a *β*-glucose unit in the molecule of **1** was evident from the chemical shift and coupling constant of the anomeric proton at δ_H 4.28 (d, $J = 7.5$ Hz) and the chemical shifts of six carbon resonances observed at δ_C 100.8 (CH), 73.4 (CH), 76.1 (CH), 69.9 (CH), 75.9 (CH), and 61.0 (CH_2).[9] Interpretation of the observed 1D ^1H NMR data and 2D $^1H-^1H$ COSY, HMQC, and HMBC correlations related to the rest of the 15 carbon resonances of compound **1** suggested that this isolate is a sesquiterpene *β*-glucoside with a gross structure as shown in Figure 1. Both the ^1H and ^{13}C NMR data (Tables 1 and 2) of the aglycone of compound **1** were found to be closely comparable to the published values for (*E*,*E*)-3*β*,9*β*-dihydroxy-6*β*H,11*β*H-germacra-1(10),4-dien-12,6-lactone.[10] The location of the glucose unit was assigned at C-3 unambiguously on the basis of the observed HMBC correlations from H-3 at δ_H 4.41 to C-1″ at δ_C 100.8 and from H-1″ at δ_H 4.28 to C-3 at δ_C 83.7. The relative configuration of **1** was confirmed by analysis of the chemical shifts, coupling constants, and NOE correlations observed for the key ^1H and ^{13}C NMR resonances. The relatively upfield chemical shifts of the allylic methyls (C-14, δ_C 10.3; C-15, δ_C 11.6) suggested that both double bonds in the molecule of **1** are in the *E*-form.[10] The large coupling constants for H-5 (4.77, brd, $J = 10.2$ Hz) and H-6 (4.88, dd, $J = 9.6$, 8.7 Hz) suggested a *trans*-lactone junction.[10] The resonance of C-15 was observed in a relatively upfield position at δ_C 11.6 and indicated that the glucopyranoxy functionality at C-3 is in the *β*-orientation.[10] The chemical shift and coupling constant of H-9 (4.00, brd, $J = 8.4$ Hz) suggested that OH-9 is *β*-oriented in the molecule of **1**. In NOE experiments, irradiation of H-11 led to an enhancement of H-6 (+6.58%), while irradiation of H-9 led to an enhancement of H-7 (+5.86%). These data were used to determine an α-orientation for H-7 and H-9 and a *β*-orientation for H-6 and H-11. In order to determine the absolute configuration of the *β*-glucose unit, compound **1** was first hydrolyzed and then derivatized by a method described previously.[11] GC analysis of its trimethylsilyl D-cysteine derivative revealed the presence of D-glucose. Therefore, compound **1** was elucidated as (*E*,*E*)-3*β*-*O*-*β*-D-glucopyranosyl-9*β*-hydroxy-6*β*,7α,11*β*H-germacra-1(10),4-dien-12,6-olide and named scorzoaustriacoside. To our knowledge, compound **1** is the first germacrane-type sesquiterpene lactone to have been isolated from the genus *Scorzonera*.[2]

Compound **2** was assigned a molecular formula of $C_{20}H_{27}O_5N$ from the sodiated molecular ion observed at *m/z* 384.1785 in its HRESIMS, requiring eight degrees of unsaturation. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) as well as the observed $^1H-^1H$ COSY, HMQC, and HMBC correlations (Figure 1) suggested that compound **2** is a guaiane-type sesquiterpene derivative structurally related to 11*β*,13-dihydrozaluzanin.[12] In addition to the resonances assigned to the skeleton of the sesquiterpene, the ^{13}C and DEPT NMR data indicated the presence of one more carbonyl carbon at δ_C 173.6 (C-5′), one more oxygenated methine carbon at δ_C 70.8 (C-2′), and three methylenes at δ_C 54.6 (C-1′), 28.8 (C-4′), and 23.5 (C-3′) in the molecule of **2**. Further interpretation of the observed ^1H (Table 1) and 2D NMR data (Figure 1) related to these carbon resonances was used to determine the presence of an aminomethyl-*γ*-butyrolactone moiety.[13] By considering of the chemical shifts of both C-13 (δ_C 53.3) and C-1′ (δ_C 54.6) and the elemental composition determined from the HRMS, it could be concluded that the aminomethyl-*γ*-butyrolactone moiety and the sesquiterpene unit are connected between C-13 and C-1′ through a nitrogen atom. This was confirmed by an HMBC correlation observed from H-13a at δ_H 3.40 to C-1′ and C-12 at δ_C 178.9. The relative configuration of **2** was determined by the key NOE correlations observed. The relative configurations of H-3 and CH_2-13 were assigned by the strong NOESY correlations from H-6 to H-3 and H-1 and from CH_2-13 to H-1, H-15, and H-7, respectively. This was confirmed by a 1D NOE experiment, in which irradiation of H-3 led to the enhancements of H-5 (+0.78%) and H-7 (+2.02%). Consequently, compound **2** was elucidated as 1αH,5αH,6*β*H,7αH,11*β*H-3*β*-hydroxy-11α-[(tetrahydro-5-oxo-fu-

* To whom correspondence should be addressed. Tel: +86-13909445175. Fax: +86-931-8912582. E-mail: zhuy@lzu.edu.cn.
† College of Chemistry and Chemical Engineering.
‡ Radiochemistry Laboratory.

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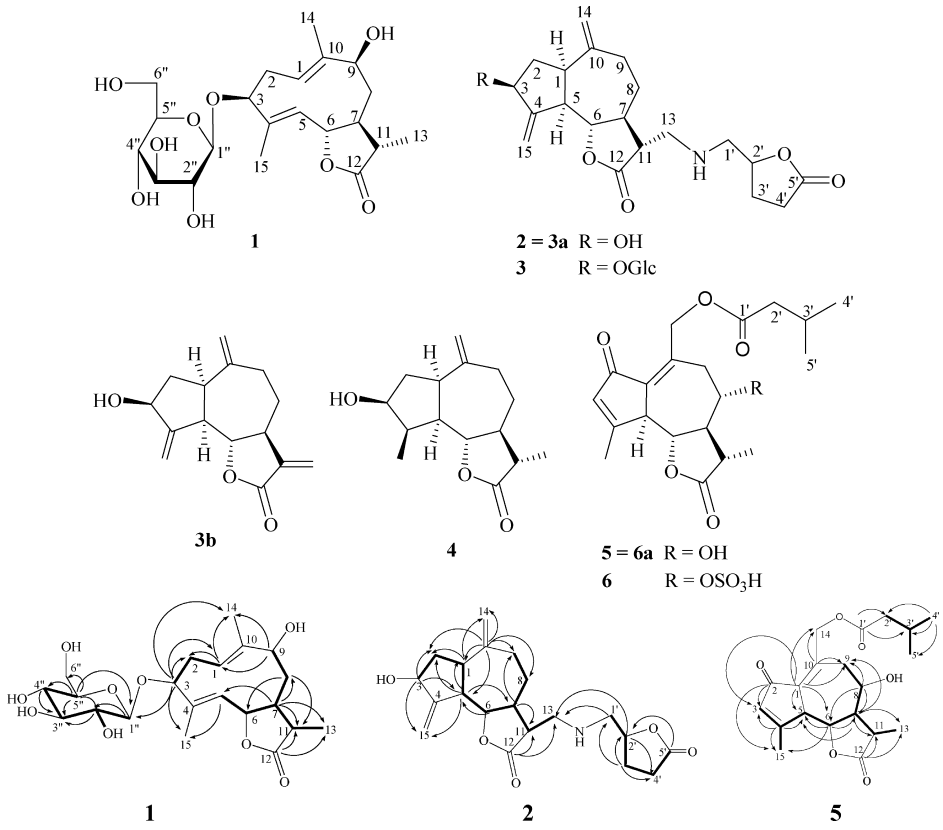

Figure 1. Key COSY (bold) and HMBC (C → H) correlations for **1**, **2**, and **5**.

Table 1. ^{1}H NMR Spectroscopic Data for **1**−**4** [δ_H, mult. (*J* in Hz)][a]

position	**1**[b]	**2**[b]	**2**[c]	**3**[b]	**4**[d]
1	5.05 brdd (9.0, 6.9)	2.72 brdd (10.0, 6.8)	2.75 dd (10.2, 8.7)	2.85 dd (10.0, 8.0)	2.63 ddd (12.9, 3.6, 3.3)
2a	2.36 brdd (8.4, 8.1)	2.14 ddd (13.6, 7.6, 6.0)	2.16 ddd (12.9, 8.4, 6.0)	2.28 ddd (14.0, 8.0, 7.6)	1.99 ddd (12.3, 9.3, 6.9)
2b	2.36 brdd (8.4, 8.1)	1.44 ddd (13.2, 7.8, 7.2)	1.50 ddd (12.3, 8.1, 7.8)	1.79 ddd (14.1, 6.3, 6.0)	1.92−1.90 m
3	4.41 dd (8.7, 7.2)	4.38 dd (7.6, 8.0)	4.33 t (8.1)	4.55 dd (6.4, 7.2)	4.23 ddd (6.9, 6.0, 4.4)
4					2.36 t (6.9)
5	4.77 brd (10.2)	2.77 dd (10.0, 9.2)	2.75 dd (10.2, 8.7)	2.77 dd (10.0, 8.0)	2.71 brd (10.5)
6	4.88 dd (9.6, 8.7)	4.11 t (9.2)	3.98 dd (9.9, 9.0)	4.23 dd (10.0, 9.6)	4.11 dd (10.8, 9.9)
7	1.82−1.93 m	2.11 brd (7.6)	2.36 brdd (12.9, 10.5)	2.21 brdd (12.8, 11.6)	2.30 ddd (12.9, 7.8, 6.9)
8α	1.82−1.93 m	1.87 brd (9.6)	2.06 dd (11.7, 4.0)	1.93−1.97 m	2.08 ddd (13.2, 4.2, 3.6)
8*β*	1.75 brd (12.3)	1.23 dd (13.8, 8.4)	1.26 brd (8.1)	1.31 dd (12.0, 10.0)	1.31 td (12.9, 4.2)
9*β*	4.00 brd (8.4)	2.22−2.31 m	2.36 brdd (12.9, 10.5)	2.36−2.39 m	1.99 ddd (12.3, 9.3, 6.9)
9α		1.76 ddd (10.1, 7.6, 4.8)	1.90 ddd (12.3, 9.3, 6.6)	2.02−2.05 m	1.81 ddd (12.3, 8.5,4.5)
10					
11	2.47 dq (10.8, 6.9)	2.88 td (11.2, 4.0)	2.58 ddd (8.7, 6.0, 5.0)	2.95 ddd (11.1, 8.7, 6.0)	2.22 ddd (10.5, 7.2, 3.3)
12					
13a	1.12 d (7.2)	3.40 dd (12.4,11.2)	3.03 dd (11.1, 6.6)	3.56 dd (12.4, 6.0)	1.22 d (7.2)
13b		3.27 dd (12.4, 4.0)	2.83 dd (13.8, 9.0)	3.30 d (6.0)	
14a	1.40 s	4.77 s	4.84 s	4.85 brs	5.01 s
14b		4.70 s			4.96 s
15a	1.64 s	5.04 s	5.11 s	5.25 brs	0.95 d (6.9)
15b		5.02 s	5.04 s	5.20 brs	
1′a		3.65 brt (7.6)	3.70−3.60 m	3.66 brd (3.7)	
1′b		3.08 brdd (13.2, 7.6)	3.01 dd (6.6, 4.5)	3.10 brd (6.9)	
2′		3.85 dd (7.2, 3.9)	3.31 dd (4.5, 5.1)	3.92 dd (10.8, 6.9)	
3′a		1.95−2.00 m	2.00 brd (6.6)	2.35−2.39 m	
3′b		1.81 brdd (9.3, 3.6)	1.82−1.89 m	1.88 dd (8.8, 6.0)	
4′a		2.22−2.31 m	1.92, brdd (12.3, 6.6)	2.28−2.36 m	
4′b		1.95−2.00 m	1.70, brdd (8.7, 7.8)	2.02−2.05 m	
Glc-1″	4.28 d (7.5)			4.47 d (8.0)	
Glc-2″	3.20 dd (9.0, 7.5)			3.15 dd (9.2, 8.0)	
Glc-3″	3.30 brd (7.8)			3.35 dd (9.2, 7.2)	
Glc-4″	3.30 brd (7.8)			3.22 dd (9.6, 9.2)	
Glc-5″	3.37 dd (8.7, 8.1)			3.35 dd (9.2, 7.2)	
Glc-6″a	3.80 brd (12.3)			3.92 dd (12.8, 10.0)	
Glc-6″b	3.59 dd (12.0, 3.9)			3.47 dd (12.8, 11.2)	

[a] Assignments were made using ^{1}H−^{1}H COSY, HMBC, and NOE experiments. [b] Recorded at 400 MHz in D_2O. [c] Recorded at 300 MHz in DMSO-d_6. [d] Recorded at 300 MHz in $CDCl_3$.

Table 2. ^{13}C NMR Spectroscopic Data (75 MHz) for **1**−**4** (δ_C, mult.)

position	**1**[a]	**2**[a]	**2**[b]	**3**[a]	**4**[c]
1	126.5 CH	42.7 CH	42.8 CH	43.6 CH	41.5 CH
2	31.7 CH_2	37.3 CH_2	38.8 CH_2	36.5 CH_2	39.6 CH_2
3	83.7 CH	72.5 CH	72.2 CH	81.1 CH	74.0 CH
4	140.5 qC	152.8 qC	155.1 qC	149.7 qC	40.5 CH
5	126.5 CH	46.1 CH	48.9 CH	49.0 CH	47.4 CH
6	81.9 CH	85.8 CH	84.4 CH	85.3 CH	83.3 CH
7	50.1 CH	48.1 CH	45.2 CH	46.5 CH	51.7 CH
8	35.4 CH_2	31.1 CH_2	32.3 CH_2	31.0 CH_2	33.0 CH_2
9	79.1 CH	35.1 CH_2	37.2 CH_2	34.4 CH_2	34.9 CH_2
10	139.4 qC	149.6 qC	150.5 qC	149.5 qC	148.5 qC
11	41.9 CH	42.4 CH	45.6 CH	43.0 CH	42.4 CH
12	182.8 qC	178.9 qC	177.9 qC	178.9 qC	179.1 qC
13	12.2 CH_3	53.3 CH_2	53.8 CH_2	54.6 CH_2	13.5 CH_3
14	10.3 CH_3	113.0 CH_2	113.0 CH_2	113.6 CH_2	112.1 CH_2
15	11.6 CH_3	109.5 CH_2	107.8 CH_2	113.6 CH_2	8.3 CH_3
1′		54.6 CH_2	52.1 CH_2	53.3 CH_2	
2′		70.8 CH	67.5 CH	70.9 CH	
3′		23.5 CH_2	24.2 CH_2	23.5 CH_2	
4′		28.8 CH_2	29.3 CH_2	28.9 CH_2	
5′		173.6 qC	174.3 qC	173.7 qC	
Glc-1″	100.8 CH			101.5 CH	
Glc-2″	73.4 CH			73.4 CH	
Glc-3″	76.1 CH			76.0 CH	
Glc-4″	69.9 CH			69.9 CH	
Glc-5″	75.9 CH			76.0 CH	
Glc-6″	61.0 CH_2			61.0 CH_2	

[a] Measured in D_2O. [b] Measured in DMSO-d_6. [c] Measured in $CDCl_3$.

ran-2-yl)methylamino)]methylguaia-4(15),10(14)-dien-12,6-olide and named scorzoaustriacin.

A molecular formula of $C_{26}H_{37}O_{10}N$ was assigned for compound **3** from the protonated molecular ion observed at *m/z* 524.2499 in the HRESIMS. Comparison of the ^1H and ^{13}C NMR data of **3** with those of **2** (Tables 1 and 2) suggested that **3** is a β-glucoside of **2**. The glucose was suggested to be located at C-3, since the resonance for this carbon in **3** was downfield shifted significantly (δ_C 81.1) compared to that observed in **2** (δ_C 72.5).[14] This was confirmed by HMBC correlations observed from H-1″ to C-3 and from H-3 to C-1″. The glucose was determined to be in the D-form by the same method as described for compound **1**. Compounds **3a** and **3b** were obtained from **3** via acid hydrolysis and were identified as compound **2** and zaluzanin C[12] by ^1H NMR data. Therefore, compound **3** was assigned as scorzoaustriacin 3-*O*-β-D-glucoside. The unusual aminomethyl-γ-butyrolactone moiety may be synthesized biogenetically from naturally occurring 4-hydroxymethyl-γ-butyrolactone,[15,16] possibly formed via the naturally abundant glutamic acid as a precursor.[13,16,17] Zaluzanin C reacted with an aminomethyl-γ-butyrolactone to give **2** through 1,4-nucleophilic addition of primary amines under acid catalysis.

Compound **4** was assigned a molecular formula of $C_{15}H_{22}O_3$ from the HRESIMS (observed *m/z* 273.1463 $[M + Na]^+$). The ^1H and ^{13}C NMR spectroscopic data of **4** (Tables 1 and 2) were very close to those of dihydroestafiatol.[12] The major differences between the ^1H and ^{13}C NMR data of these two compounds were the chemical shifts of both H-15 and C-15. In comparison with the H-15 (δ_H 1.24) and C-15 (δ_C 14.1) resonances of dihydroestafiatol,[12] the resonances of H-15 (δ_H 0.95) and C-15 (δ_C 8.3) were upfield shifted significantly in **4**. This suggested that the difference between compound **4** and dihydroestafiatol is at C-4. A β-orientation of CH_3-15 in **4** was evident from the enhancement of H-15 (+1.99%) by irradiation of H-6 in a 1D NOE experiment. In addition, the specific rotation sign observed for **4** ($[\alpha]_D^{20}$ −31; *c* 1.44, Me_2CO) is opposite that reported for dihydroestafiatol ($[\alpha]_D^{25}$ +25; *c* 0.08, $CHCl_3$).[12] Consequently, compound **4** was elucidated as 4-*epi*-dihydroestafiatol.

Compound **5** gave a molecular formula of $C_{20}H_{26}O_6$, as deduced from the HRESIMS (observed *m/z* 363.1804 $[M + H]^+$), requiring eight degrees of unsaturation. The ^1H and ^{13}C NMR data (Table 3) were closely comparable to those of desacetylmatricarin.[18] Further interpretation of these data and the correlations observed in the ^1H−^1H COSY, HMQC, and HMBC spectra revealed that compound **5** is a matricarin-type guaianolide possessing an isovaleroyloxy group (Figure 1).[19] The isovaleroyloxy functionality was assigned at C-14 on the basis of the observed HMBC correlations from H-14a at δ_H 5.53 and H-14b at δ_H 5.33 to C-1′ at δ_C 173.0 and C-1 at δ_C 135.8. The hydroxy group at C-8 was assigned with an α-orientation by the NOESY correlations observed from H-8 at δ_H 3.74 to H-11 and H-9b and from H-9a at δ_H 2.64 to H-5, H-7, and H-13. Consequently, compound **5** was elucidated as 5αH,6βH,7αH,11βH-8α-hydroxy-14-isovaleroxy-1(10),3-dien-2-oxo-guaian-12,6-olide and named 14-isovaleroxyscorzoaustricin.

The ^1H and ^{13}C NMR data of compound **6** were very similar to those of **5** (Table 3). The evident differences were the chemical shifts of H-7, H-8, and H-9; and C-7, C-8, and C-9. This information, in combination with the consideration of a molecular formula of $C_{20}H_{26}O_9S$ (a SO_3 unit greater than that of **5**), as determined from the HRESIMS (observed *m/z* 443.1379 $[M + H]^+$), suggested that compound **6** is the sulfate form of **5**. The EIMS showed very weak fragment ions at *m/z* 362 $[M - SO_3]^+$ and 344 $[M - H_2SO_4]^+$, which were consistent with the presence of a sulfate functionality in **6**. The IR spectrum also exhibited strong absorption bands for a sulfate group (1259, 1220, and 1116 cm^{-1}).[20] Solvolysis of **6** gave compound **5** (**6a**), which confirmed the structure of **6**. Therefore, the structure of this compound was concluded to be 14-isovaleroxyscorzoaustricin sulfate. Natural sulfates have been found to exist in a wide variety of marine organisms, especially from sponges and echinoderms, such as alkane, meroterpenoid, polyhydroxy, sesterterpenoid, steroid, and triterpenoid sulfates. However, very few natural sulfates have been found in terrestrial plants, especially sulfated sesquiterpenoids.[21,22]

The known compounds zaluzanin C,[12] glucozaluzanin C,[23] dehydrocostuslactone,[24] 11β,13-dihydrozaluzanin,[12] and diacetoxyisolippidiol[25] were identified by comparison of their physical and spectroscopic data with those reported in the literature.

Four compounds obtained in this investigation, **2**, **3**, **5**, and **6**, were evaluated for their cytotoxic activity against four human tumor cell lines, namely, K562, K562/ADM, BGC-823, and Hep-G2. Among these compounds, only compound **2** was found to be

Table 3. NMR Spectroscopic Data for Compounds **5** and **6**[a]

	5[b]		**6**[c]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	135.8 qC		136.0	
2	194.4 qC		194.4	
3	135.4 CH	6.20 brs	134.7	6.18 s
4	171.5 qC		172.0	
5	51.5 CH	3.45 d (10.1)	51.0	3.70 d (9.9)
6	80.5 CH	3.70 dd (10.1, 10.0)	80.7	3.89 t (9.9)
7	61.5 CH	2.17 ddd (10.0, 9.6, 1.6)	59.7	2.37 dd (10.5, 10.2)
8	70.0 CH	3.74 ddd (10.0, 3.8, 1.4)	73.9	4.33 dd (10.2, 9.0)
9	41.9 CH_2	2.64 dd (14.0, 10.0)	38.1	3.12−3.18 m
		2.59 dd (14.0, 3.8)		2.54 dd (13.8, 11.4)
10	141.7 qC		142.7	
11	41.2 CH	2.57 dq (9.6,7.0)	40.5	2.76 dq (11.7, 6.9)
12	177.3 qC		177.4	
13	15.5 CH_3	1.46 d (7.0)	14.9	1.38 d (6.9)
14	62.2 CH_2	5.53 d (13.6)	61.4	5.47 d (13.5)
		5.33 d (13.6)		5.36 d (13.5)
15	20.0 CH_3	2.33 s	19.1	2.30 s
i-Val-1′	173.0 qC		173.0	
i-Val-2′	43.2 CH_2	2.25 d (7.0)	42.6	2.33 dd (7.2, 6.6)
i-Val-3′	25.7 CH	2.16 tq (7.0, 6.6)	25.3	2.11 tq (7.2, 6.9)
i-Val-4′	22.4 CH_3	0.97 d (6.6)	21.8	0.94 d (6.6)
i-Val-5′	22.4 CH_3	0.97 d (6.6)	21.8	0.94 d (6.6)

[a] ^{1}H and ^{13}C chemical shifts were assigned by ^{1}H−^{1}H COSY, HMBC, NOE, and NOESY data. [b] Recorded in $CDCl_3$ at 400 and 100 MHz for ^{1}H and ^{13}C NMR, respectively. [c] Recorded in acetone-d_6 at 300 and 75 MHz for ^{1}H and ^{13}C NMR, respectively.

marginally active, with an IC_{50} value of 11.3 μM, for the K562 human erythroleukemia cell line.

Experimental Section

General Experimental Procedures. Melting points were measured using an uncorrected X-4 Digital Display micromelting point apparatus. Optical rotations were obtained on a Perkin-Elmer 341 digital polarimeter in $CHCl_3$. UV spectra were run in MeOH on a Shimadzu UV-260 spectrophotometer. IR spectra were carried out a Nicolet FTIR-360 or a Bio-Rad FTS165-IR spectrometer. ^{1}H NMR (1D, ^{1}H−^{1}H COSY, NOE) and ^{13}C NMR (1D, HMQC, HMBC, NOESY) spectra were recorded on Bruker AM-400 or Varian Mercury-300 NMR spectrometers, and the chemical shifts are given as δ values with TMS as the internal standard. HRESIMS were recorded on a Bruker APEX II47e mass spectrometer. EIMS and FABMS were measured on HP5988a GC-MS and VG-ZAB-MS mass spectrometers at 70 eV, respectively. GLC was carried out on a TRACE DSQ GC-MS instrument. Solvents were distilled prior to use, and spectroscopic grade solvents were employed. Column chromatography was carried out on silica gel (200−300 mesh and Type 60) and TLC on silica gel (GF_{254}, 10−40 μm), with both materials supplied by Qingdao Marine Chemical Co.

Plant Material. The roots of *Scorzonera austriaca* were collected at the North Mountains of Huzhu County, Qinghai Province, People's Republic of China, in August 1999, and identified by Prof. Fu-Jia Zhang, School of Life Science, Lanzhou University. A voucher specimen (Sa19990801) has been deposited at the State Key Laboratory of Applied Organic Chemistry, Lanzhou University.

Extraction and Isolation. The air-dried roots of *S. austriaca* (3.5 kg) were powdered and first extracted with acetone (4 × 20 L), then with 95% ethanol (2 × 20 L). The extracts were concentrated under reduced pressure to yield dried residues. The dried acetone residue (182 g) was chromatographed over a silica gel column, using petroleum ether (60−90 °C)−acetone mixtures of increasing polarity, and finally washed with MeOH. The 6:1 petroleum ether−acetone fraction was subjected to further chromatographic separation with petroleum ether−EtOAc, then $CHCl_3$−acetone, to yield **4** (15 mg). The 1:1 petroleum ether−acetone fraction was subjected to further chromatographic separation with $CHCl_3$−MeOH, then petroleum ether−acetone and petroleum ether−EtOAc−MeOH, to give **5** (15 mg). The 1:2 petroleum ether−acetone fraction was chromatographed with petroleum ether−acetone, then $CHCl_3$−MeOH, to give **6** (25 mg). The dried ethanol residue (400 g) was suspended in distilled water and extracted with *n*-butanol (4 × 500 mL). The water layer was evaporated under reduced pressure to give a residue (300 g), which was subjected to column chromatography on a resin (D-3520), with EtOH−H_2O mixtures of increasing polarity used for elution. The 100% H_2O fraction was subject to further chromatographic separation using Me_2CO−MeOH, then CH_2Cl_2−MeOH and EtOAc−MeOH, to yield **1** (20 mg) and **3** (35 mg). The 5% EtOH−H_2O fraction was subjected to chromatographic separation with Me_2CO−MeOH to yield **2** (25 mg).

Compound 1: colorless oil; $[\alpha]_D^{20}$ +194 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.8) nm; IR ν_{max} (film) 3355, 1749, 1638, 1076, 1048, 965 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 428 $[M]^+$; HRESIMS *m/z* 446.2376 $[M + NH_4]^+$ (calcd for $C_{21}H_{36}O_9N$, 446.2385).

Compound 2: colorless crystals; mp 180−181 °C (MeOH); $[\alpha]_D^{20}$ −3 (*c* 0.60, MeOH); IR (KBr) ν_{max} 3401, 3077, 1749, 1645 1452, 1373, 1322, 1201, 977, 910 cm^{-1}; UV (MeOH) λ_{max} (log ε) 215 (3.0) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 362 (37), 316 (15), 256 (35), 117 (18), 53 (100); FABMS *m/z* 362 $[M + H]^+$, 316, 256, 116; HRESIMS *m/z* 362.1964 $[M + H]^+$ (calcd for $C_{20}H_{28}O_5N$, 362.1962), 384.1785 $[M + Na]^+$ (calcd for $C_{20}H_{27}O_5NNa$, 384.1781).

Compound 3: colorless crystals; mp 148−149 °C (MeOH); $[\alpha]_D^{20}$ −91 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.0) nm; IR (KBr) ν_{max} 3519, 3357, 3256, 1751, 1645, 1036, 979, 899 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z* 524.2499 $[M + H]^+$ (calcd for $C_{26}H_{38}O_{10}N$, 524.2490).

Compound 4: colorless gum; $[\alpha]_D^{20}$ −11 (*c* 0.33, $CHCl_3$), UV (MeOH) λ_{max} (log ε) 215 (2.6) nm; IR (KBr) ν_{max} 3400, 1763, 1639, 1066, 1027, 987, 913, 734 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 250 (0.7) $[M]^+$, 232 (5) $[M - H_2O]^+$, 177 (95), 152 (100); HRESIMS *m/z* 273.1463 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1461).

Compound 5: colorless gum; $[\alpha]_D^{20}$ −8 (*c* 0.38, Me_2CO); UV (MeOH) λ_{max} (log ε) 210 (4.2), 241 (4.6) nm; IR (KBr) ν_{max} 3463, 1778, 1734, 1687, 1643, 1615, 1375, 1291, 1188, 1163, 1116, 986 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m/z* 362 $[M]^+$, 260 (13), 57 (100); HRESIMS *m/z* 363.1804 $[M + H]^+$ (calcd for $C_{20}H_{28}O_6$, 363.1802).

Compound 6: colorless crystal; mp 158−159 °C (MeOH); $[\alpha]_D^{20}$ −31 (*c* 1.44, Me_2CO); UV (MeOH) λ_{max} (log ε) 251 (3.4) nm; IR ν_{max} (KBr) 3472, 1779, 1727, 1689, 1645, 1616, 1459, 1259, 1220, 1162, 1116, 1065, 1015, 961, 817 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; EIMS *m/z* 362 (0.7), 344 (0.7), 278 (39) $[M - HSO_3 - C_5H_9O]^+$, 260 (0.7) $[M - SO_3 - C_5H_{10}O_2]^+$, 41 (100); HRESIMS *m/z* 443.1379 $[M + H]^+$ (calcd for $C_{20}H_{27}O_9S$, 443.1370), $[M + Na]^+$ at *m/z* 465.1186.

Acid Hydrolysis of 1. Compound **1** (5 mg) in 5 mL of 2 N CF_3COOH was stirred for 5 h at 95 °C. On cooling, the reaction mixture was extracted with $CHCl_3$ three times to afford an aqueous layer and organic layer. The aqueous phase was concentrated under reduced

pressure to give a residue, which was purified on a Sephadex LH-20 column eluted with CH_3Cl_3−MeOH (2:1), to afford a sugar fraction. The sugar fraction was dissolved in pyridine (0.5 mL each) and stirred with D-cysteine methyl ester hydrochloride (0.08 M) in pyridine (0.75 mL) before 1-trimethylsilylimidazole (0.5 mL) was added to the mixture, using the same procedures as in a previous report.[11] The mixture was partitioned between *n*-hexane and H_2O (0.5 mL each). The *n*-hexane fraction was analyzed by GC-MS using a DB-1701 column (30 m × 0.25 mm × 0.25 μm). The temperatures of the transfer line and source were 280 and 250 °C, respectively. A temperature gradient system was used for the oven, starting at 80 °C for 1 min and increasing up to 280 °C, at a rate of 20 °C/min. D-Glucose (t_R 13.74 min) was identified as the sugar moiety of **1** by comparing its retention time with those of authentic samples of D-glucose (t_R 13.74 min) and L-glucose (t_R 13.65 min), after treatment in the same manner.

Acid Hydrolysis of 3. Compound **3** (10 mg) was first hydrolyzed, then derivatized and analyzed, as described for **1**. D-Glucose was detected by GC analysis (t_R 13.74). The organic layer of **3** was dried completely under reduced pressure and chromatographed on a silica gel column, eluted with CH_2Cl_2−Me_2CO mixtures, to give **3a** (2 mg) and **3b** (2 mg). They were identified as compound **2** (**3a**) and zaluzanin C (**3b**)[12] by ^{1}H NMR and EIMS, respectively. **3a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 5.44 (1H, s, H-15a), 5.38 (1H, s, H-15b), 4.96 (1H, s, H-14a), 4.93 (1H, s, H-14b), 4.56 (1H, m, H-3), 4.16 (2H, dd, J = 13.3, 7.2 Hz, H-1′a, H-2′), 4.03 (1H, t, J = 9.9 Hz, H-6), 3.34 (1H, m, H-13a). 3.07 (1H, J = 13.9, 3.5 Hz, H-13b), 3.02−2.80 (2H, m, H-11, H-1′b), 2.58−2.42 (3H, m, H-1, H-5, H-4′a), 2.38−2.20 (2H, m, H-2a, H-7), 2.08−2.00 (3H, m, H-9a, H-3′a, H-4′b), 1.99−1.70 (3H, m, H-8a, H-9b, H-3′b), 1.70−1.50 (1H, m, H-2b), 1.40−1.20 (1H, m, H-8b); EIMS *m*/*z* 361 (3.8), 229 (100), 215 (32), 159 (40), 43 (52). **3b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ_H 6.22 (1H, d, J = 3.3 Hz, H-13a), 5.49 (1H, d, J = 3.0 Hz, H-13b), 5.47 (1H, s, H-15a), 5.33 (1H, s, H-15b), 5.01 (1H, s, H-14a), 4.95 (1H, s, H-14b), 4.57 (1H, m, H-3), 4.10 (1H, t, J = 9.6 Hz, H-6), 2.92 (1H, dd, J = 9.6, 8.1 Hz, H-1), 2.86 (2H, brd, J = 10.5 Hz, H-5, H-7), 2.50 (1H, brd, J = 12.9 Hz, H-9a), 2.33 (1H, dd, J = 13.5, 6.9 Hz, H-2a), 2.15−2.05 (1H, m, H-9b), 1.78 (1H, dd, J = 12.9, 6.9 Hz, H-2b), 1.69 (1H, m, H-8a), 1.38−1.20 (1H, m, H-8b); EIMS *m*/*z* 246 (15), 228 (23), 218 (29), 200 (47), 177 (59), 175 (38), 152 (55), 129 (86), 105 (75), 91(100).

Solvolysis of 6. Compound **6** (10 mg) was dissolved in a mixture of dioxane−pyridine (1:1, 5 mL) and heated at 130 °C under reflux for 3 h. After solvolysis, the solution was neutralized with 5% $NaHCO_3$ and extracted with EtOAc three times. The organic layer was dried completely under reduced pressure, and the residue was purified by column chromatography on silica gel, with petroleum ether−acetone (5:1), to afford the desulfated derivative **6a** (3 mg), which was elucidated as **5** from its ^{1}H NMR ($CDCl_3$, 300 MHz): δ_H 6.20 (1H, s, H-3), 5.55 (1H, d, J = 13.5 Hz, H-14a), 5.31 (1H, d, J = 13.5 Hz, H-14b), 3.70 (2H, t, J = 9.9 Hz, H-6, H-8), 3.43 (1H, d, J = 9.9 Hz, H-5), 2.62−2.58 (1H, m, H-9a), 2.55 (1H, brd, J = 9.3 Hz, H-9), 2.33 (2H, s, H-15, H-2′), 2.25 (1H, d, J = 6.9 Hz, H-11), 2.17 (1H, d, J = 10.5 Hz, H-7), 2.13−2.09 (1H, m, H-3′), 1.46 (1H, d, J = 6.9 Hz, H-13), 0.97 (2H, d, J = 6.3 Hz, H-4′, H-5′).

The anion SO_4^{2-} was confirmed by precipitation with $BaCl_2$. The aqueous layer was concentrated to about 10 mL, and then a solution of 0.1 M $BaCl_2$ was added. The precipitate was collected by centrifugation, since the white precipitate was not soluble on addition of 2 M HCl solution, which was confirmed to be $BaSO_4$.

Bioassay. Cytotoxic assays of compounds **2**, **3**, **5**, and **6** against the K562 human erythroleukemia, K562/ADM human erythroleukemia adriamycin-resistant subline, BGC-823 human stomach carcinoma, and Hep-G2 human hepatoma cell lines were carried out using the MTT method, as described previously.[26]

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Supporting Information Available: 1D and 2D NMR and HRES-IMS spectra of **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Editorial Committee for Flora of the Chinese Academy of Science. In *Flora of China*; Ling, Y., Shih, C., Eds.; Science Press: Beijing, People's Republic of China, 1997; Vol. 80 (1), pp 13−36.
(2) Zidorn, C. *Phytochemistry* **2008**, *69*, 2270–2296.
(3) Paraschos, S.; Magiatis, P.; Kalpoutzakis, E.; Harvala, C.; Skaltsounis, A.-L. *J. Nat. Prod.* **2001**, *64*, 1585–1587.
(4) Zidorn, C.; Ellmerer, E. P.; Sturm, S.; Stuppner, H. *Phytochemistry* **2003**, *63*, 61–67.
(5) Northwest Plateau Institute of Biology, Chinese Academy of Sciences. In *The Economic Flora of Qinghai*; Gao, B.-C., Ed.; Qinghai People's Press: Xining, People's Republic of China, 1987; p 652.
(6) Jiang, T.-F.; Wang, Y.-H.; Lv, Z.-H.; Yue, M.-E. *J. Pharm. Biomed. Anal.* **2007**, *43*, 854–858.
(7) Li, J.; Wu, Q.-X.; Shi, Y.-P.; Zhu, Y. *Chin. Chem. Lett.* **2004**, *15*, 1309–1310.
(8) Zhu, Y.; Wu, Q.-X.; Hu, P.-Z.; Wu, W.-S. *Food Chem.* **2009**, *114*, 1316–1320.
(9) Kotsos, M. P.; Aligiannis, N.; Myrianthopoulos, V.; Mitaku, S.; Skaltsounis, L. *J. Nat. Prod.* **2008**, *71*, 847–851.
(10) Zhang, H.; Liao, Z.-X.; Yue, J.-M. *Helv. Chim. Acta* **2004**, *87*, 976–982.
(11) Li, W.; Fu, H.-W.; Bai, H.; Sasaki, T.; Kato, H.; Koike, K. *J. Nat. Prod.* **2009**, *72*, 1755–1760.
(12) Kumari, G. N. K.; Masilamani, S.; Ganesh, M. R.; Aravin, S. *Phytochemistry* **2003**, *62*, 1101–1104.
(13) Pieper, B.; Traenkle, C.; Nieger, M.; Mohr, K.; Lehmann, J. *Pharmazie* **1995**, *50*, 15–21.
(14) Zidorn, C.; Ellmerer, E.-P.; Konwalinka, G.; Schwaiger, N.; Stuppner, H. *Tetrahedron Lett.* **2004**, *45*, 3433–3436.
(15) Xiong, Y.; Deng, K.-Z.; Gao, W.-Y.; Guo, Y.-G. *China J. Chin. Mater. Med.* **2008**, *33*, 909–911.
(16) Herdeis, C. *Synthesis* **1986**, 232–233.
(17) Boukouvalas, J.; Radu, I.-I. *Tetrahedron Lett.* **2007**, *48*, 2971–2973.
(18) Martinez, V. M.; Muñoz-Zamora, A.; Joseph-Nathan, P. *J. Nat. Prod.* **1988**, *51*, 221–228.
(19) Mata, R.; Rivero-Cruz, I.; Rivero-Cruz, B.; Bye, R.; Timmermann, B. N. *J. Nat. Prod.* **2002**, *65*, 1030–1032.
(20) Makarieva, T. N.; Rho, J.-R.; Lee, H.-S.; Santalova, E. A.; Stonik, V.; Shin, J. *J. Nat. Prod.* **2003**, *66*, 1010–1012.
(21) Zidorn, C.; Ellmerer, E. P.; Heller, W.; Joehrer, K.; Frommberger, M.; Greil, R.; Guggenberger, M.; Ongania, K.-H.; Stuppner, H. *Z. Naturforsch. B* **2007**, *62*, 132–134.
(22) Yoshikawa, M.; Yamaguchi, S.; Matsuda, H.; Tanaka, N.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1994**, *42*, 2430–2435.
(23) Zdero, C.; Bohlmann, F. *Phytochemistry* **1987**, *26*, 2597–2601.
(24) Neves, M.; Morais, R.; Gafner, S.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1999**, *50*, 967–972.
(25) De Heluani, C. S.; De Boggiato, M. V.; Catalán, C. A. N.; Díaz, J. G.; Gédris, T. E.; Herz, W. *Phytochemistry* **1997**, *45*, 801–805.
(26) Hussain, R. F.; Nouri, A. M. E.; Oliver, R. T. D. *J. Immunol. Methods* **1993**, *160*, 89–96.

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