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Two New Amino Acid-Sesquiterpene Lactone Conjugates from *Ixeris dentata*

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Ixeris dentata (Asteraceae) is a perennial herb which is used frequently as a Traditional Chinese Medicine for the treatment of gastroenteric troubles, diabetes, pneumonia, hepatitis and tumor.[1,2] The young shoots of the species is commonly used as a famous bitter appetizing vegetable in Korea. *I. dentata* was characterized by the presence of guaiane sesquiterpene lactones which established as chemosystematic makers.[3,4] In the previous paper, we reported the isolation of several sesquiterpene lactones from *I. dentata*.[5] As a continuation of our effort to purify minor sesquiterpenes from *I. dentata*, two new amino acid-sesquiterpene lactones (**1**, **2**), ixerisamine A (**1**) and ixerisamine B (**2**) were isolated together with twelve related sesquiterpene lactones, such as 8-*epi*-desacylcynaropicrin glucoside (**3**),[6] ixerisoside A (**4**),[7] 6-*O*-acetylixerisoside A (**5**),[5] ixerin N (**6**),[8] 6-*O*-acetyl ixerin N (**7**),[5] ixerin M (**8**),[8] tectroside (**9**),[7] 4,8-*epi*-isolipidiol (**10**),[5] 8-*epi*-isolipidiol (**11**),[9] 11β*H*-11,13-dihydro-integrifolin (**12**),[10] 8β-hydroxy-4β,15-dihydrozaluzanin C (**13**),[11] and integrifolin (**14**),[11] respectively. In this paper, we describe the isolation and structure determination of new compounds (**1**, **2**), as well as the inhibitory effects of isolated sesquiterpenes on the proliferation of four cultured human tumor cell lines such as MES-SA (human uterine carcinoma cell line), MES-SA/DX5 (multidrug resistant subline of MES-SA), HCT-15 (human colorectal adenocarcinoma cell line) and HCT15/CL02 (multidrug resistant subline of HCT15).

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −21.6 (*c* 0.15 CH_3OH). The molecular formula of **1** was established as $C_{26}H_{37}NO_{11}$ at *m/z* 540.2439 (calcd 540.2436) $[M+H]^+$ by HR-ESI-MS. All proton and carbon signals of **1** were completely assigned by the aid of two-

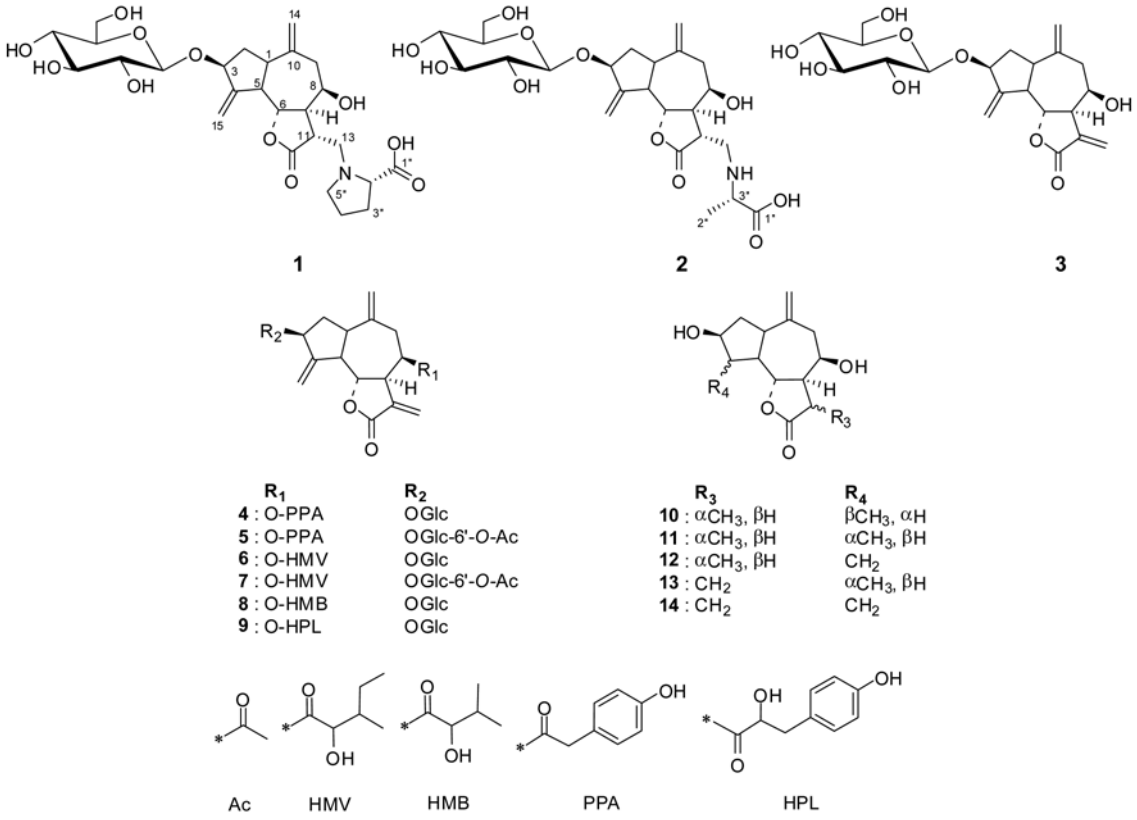


Table 1. ^{1}H NMR Spectroscopic Data of **1**-**3**[a]

Position	**1** (δ_H)	**2** (δ_H)	**3** (δ_H)
1	2.91 (1H, m)	2.86 (1H, q, *J* = 15.2, 8.0 Hz)	2.59 (1H, m)
2	2.40 (1H, m)	2.40 (1H, m)	2.43 (1H, m)
	2.20 (1H, m)		2.20 (1H, m)
3	4.87 (1H, dd, *J* = 8.0, 7.2 Hz)	4.85 (1H, dd, *J* = 7.2, 5.6 Hz)	4.88 (1H, br t, *J* = 8.4 Hz)
4			
5	2.88 (1H, m)	2.75 (1H, m)	2.86 (1H, m)
6	5.03 (1H, m)	5.00 (1H, t, *J* = 8.8 Hz)	4.88 (1H, m)
7	3.06 (1H, t, *J* = 9.6 Hz)	5.74 (1H, m)	3.14 (1H, m)
8	4.57 (1H, br s)	4.45 (1H, br s)	5.13 (1H, br s)
9	2.72 (1H, dd, *J* = 13.0, 5.0 Hz)	2.70 (1H, dd, *J* = 12.0, 4.8 Hz)	2.90 (1H, m)
	2.60 (1H, dd, *J* = 13.0, 4.4 Hz)	2.57 (1H, dd, *J* = 12.0, 4.8 Hz)	2.67 (1H, dd, *J* = 13.3, 5.3 Hz)
10			
11	3.54 (1H, m)	3.71 (1H, m)	
12			
13	3.49 (1H, dd, *J* = 12.8, 5.6 Hz)	3.48 (1H, dd, *J* = 11.2, 6.4 Hz)	6.54 (1H, d, *J* = 3.0 Hz)
	3.20 (1H, m)	3.30 (1H, dd, *J* = 11.2, 4.4 Hz)	5.63 (1H, d, *J* = 3.0 Hz)
14	5.17 (1H, s)	5.17 (1H, s)	5.21 (1H, s)
	5.03 (1H, s)	5.03 (1H, s)	5.03 (1H, s)
15	5.92 (1H, s)	5.87 (1H, s)	5.96 (1H, br s)
	5.59 (1H, s)	5.50 (1H, s)	5.74 (1H, br s)
Glc 1	5.07 (1H, d, *J* = 7.2 Hz)	5.04 (1H, d, *J* = 7.2 Hz)	5.09 (1H, d, *J* = 7.8 Hz)
Glc 2	4.10 (1H, dd, *J* = 8.8, 8.0 Hz)	4.08 (1H, dd, *J* = 7.2, 6.4 Hz)	4.11 (1H, br t)
Glc 3	4.29 (1H, t, *J* = 8.8 Hz)	4.28 (1H, dd, *J* = 8.0, 7.2 Hz)	4.31 (1H, m)
Glc 4	4.24 (1H, dd, *J* = 9.6, 8.8 Hz)	4.20 (1H, t, *J* = 8.0 Hz)	4.31 (1H, m)
Glc 5	3.99 (1H, m)	3.98 (1H, m)	4.00 (1H, m)
Glc 6	4.58 (1H, m)	4.56 (1H, dd, *J* = 10.6, 2.4 Hz)	4.45 (1H, m)
	4.38 (1H, dd, *J* = 12.0, 5.6 Hz)	4.35 (1H, dd, *J* = 10.6, 5.2 Hz)	4.28 (1H, dd, *J* = 12.0, 5.4 Hz)
1"			
2"	3.60 (1H, m)	1.58 (1H, d, *J* = 6.4 Hz)	
3"	2.12 (2H, m)	3.81 (1H, m)	
4"	1.84 (1H, m)		
	1.67 (1H, m)		
5"	3.32 (1H, m)		
	2.68 (1H, m)		

[a]Assignments are based on HMQC, and HMBC experiments, and chemical shifts are given in ppm

dimensional NMR experiments such as COSY, DEPT, HMQC, HMBC and ROESY. All proton signals of **1** were quite similar to those of **3**. However, two proton signals assignable to 13-exomethylene of **3** were completely disappeared in ^{1}H-NMR spectrum of **1**. Instead, several new proton signals were found assignable to a proline (Table 1). These results implied that a proline was added on 13-exomethylene of **3** to produce **1**. It was supported by the HMBC correlations of H-13a (δ 3.49, dd, *J* = 12.8, 5.6 Hz) with C-12 carbonyl carbon (δ 178.4) and also by the correlation between H-13a and C-2" (δ 67.8) of proline (Figure 1). The proton signal of H-11 (δ 3.54) was observed to be correlated with H-6 (δ 5.03) by ROESY experiment, which could establish the configuration of C-11 as *R*. On the other hand, **1** was slowly converted to **3** and liberated a proline (Scheme 1) by the treatment of **1** with 5% NH_4OH at room temperature. The optical rotation of liberated proline {$[\alpha]_D^{20}$ −26 (*c* 0.065, H_2O)} suggested that the stereochemistry of the proline is a L-form.[12] By these spectroscopic and chemical evidences, the chemical structure of **1**, tentatively named as ixerisamine A, was established to be a L-proline adduct of **3**, *i.e.*, 3β-{(β-D-glucopyranosyl)-8β-hydroxy-13-(*N*-L-prolinyl)}-guaia-4(15),10(14)-dien-1α,5α,6β,7α, 11β*H*-12,6α-olide.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{20}$ −7.8 (*c* 0.165, CH_3OH). The molecular formula of **2** was established as $C_{24}H_{35}NO_{11}$ at *m/z* 514.2283 (calcd 514.2281) $[M+H]^+$ by HR-ESI-MS. The ^{1}H- and ^{13}C- NMR spectra of **2** were similar to those of **1**, except for signals due to amino acid moiety (Table 1). The HMBC correlations between H-13a (δ 3.48) and C-12 carbonyl carbon (δ 178.3) and between H-13a and C-3" (δ 58.2) of the L-alanine moiety suggested that an alanine was added on 13-exomethylene of **3** (Figure 1). The relative configuration of C-11 of **2** was also established as *R* by the observation of the correlation between H-11 with H-6 by ROESY experiment.

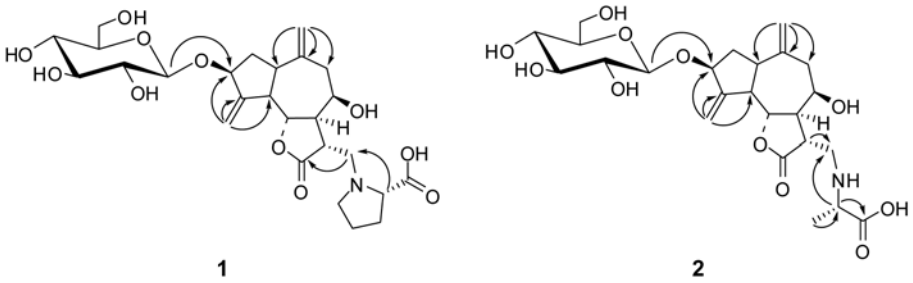
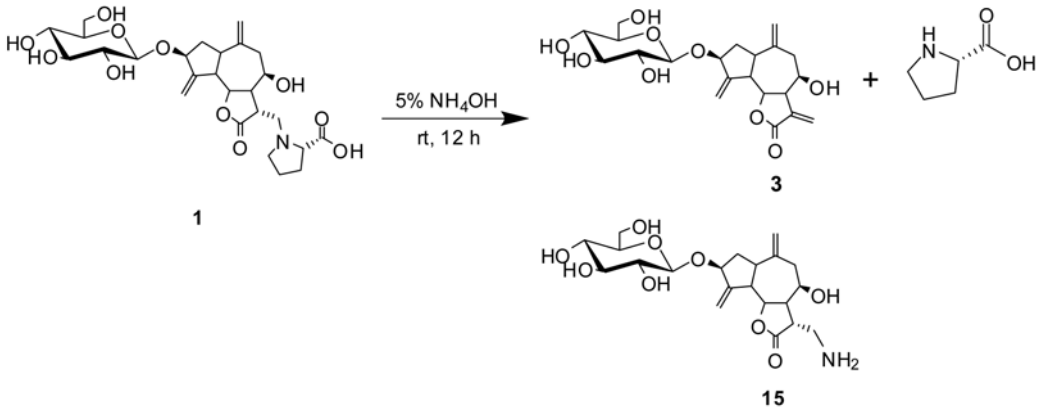


Figure 1. Key HMBC correlations of **1**, **2**.



Scheme 1. Treatment of **1** with NH_3.

Thus, the structure of **2**, tentatively named as ixerisamine B, was established to be a L-alanine adduct of **3**, *i.e.*, 3β-{(β-D-glucopyranosyl)-8β-hydroxy-13-(*N*-L-alaninyl)}-guaia-4(15), 10(14)-dien-1α,5α,6β,7α,11β*H*-12,6α-olide. Compound **1** and **2** were supposed to be produced biogenetically *via* Michael type attack of endogenous L-proline (**1**) and L-alanine (**2**) to **3** in the plant.

Some of isolated sesquiterpenes (**13**, **14**) exhibited a significant inhibitory effect on the proliferation of cultured human cancer cell lines. The ED_{50} values of them on tested cell lines were comparable with those of reference drug, etoposide. It is believed that the *exo* methylene group on the γ-lactone is essential for cytotoxicity because structural modifications such as saturation or addition to the methylene group resulted in the loss of cytotoxicity and tumor inhibition.[5] However, **1** and **2** which lack the partial structure of the unsaturated γ-lactone exhibited a relatively poor inhibition on tested tumor cells (ED_{50} > 30 μM).

Experimental Section

General Experimental Procedures. NMR spectra were obtained by a Brucker AM 300, 500 and Brucker AVANCE II 800 spectrometers using TMS as an internal standard for ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, and HMBC. HRESIMS was recorded by Applied Biosystems Mariner time-of-flight mass spectrometer with an electrospray interface. For column chromatography, Diaion HP-20, ODS (Cosmosil 140 C_{18}) were used as stationary phase. Preparative-HPLC was performed on a Futecs P-4000 system with a Shim-pack prep-ODS(H) kit column (5 μm, 20 mm × 25 cm). Isolation and purification was also carried out using a medium-pressure liquid chromatographic (MPLC) system [BUCHI pump Module C-601, silica gel 60 (230-400 mesh, Merck), ODS (Cosmosil 140 C_{18})].

Plant Material. The whole plants of *I. dentata* were collected on May 2006 at the herbarium of Korea Research Institute of Chemical Technology (KRICT) and were authenticated by us, Dr. Young Sup Kim. The voucher specimen (KR0472) was deposited at the herbarium of KRICT, Korea.

Extraction and Isolation. The air-dried whole plants (6 kg) of *I. dentata* were soaked in methanol (MeOH) (40 L × 2) at room temperature for 7 days. The MeOH extract was filtered and evaporated to dryness under reduced pressure. The concentrated extract (840 g) was suspended in 20 L of water and then extracted successively with an equal volume of dichloromethane (MC), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH), which afforded 160 g of MC fraction, 15 g of EtOAc fraction, 60 g of *n*-BuOH fraction, and 510 g of aqueous layer, respectively. The aqueous layer was poured into a Diaion HP-20 column (Φ = 5.0 × 100 cm) chromatography eluted with gradient of MeOH in H_2O (25, 50, 75 and 100%), to yield five fractions (Fr. 1-Fr. 5). Fr. 2 (6 g) was subjected to MPLC (ODS, Φ = 5.0 × 70 cm) performed by the stepwise gradient elution of aqueous MeOH, changing the ratio of water and MeOH as 9:1, 7:3, 5:5, 2:8, and 1:9 to give 6 fractions (Fr. 21-Fr. 26). Fr. 24 (623 mg) was purified by prep-HPLC (Shimpack ODS column) with 30% MeOH to give compound **1** (139 mg) and **2** (12 mg), respectively.

Ixerisamine A (1): White amorphous powder; $[\alpha]_D^{20}$

Table 2. ^{13}C NMR Spectroscopic Data of **1-3**

Position	**1** (δ_C)	**2** (δ_C)	**3** (δ_C)
1	44.3	44.6	46.8
2	38.1	38.2	40.4
3	80.5	80.5	82.8
4	151.3	151.1	153.4
5	50.6	50.6	52.3
6	78.7	79.1	80.6
7	49.1	49.9	51.8
8	64.7	64.6	67.9
9	44.3	43.9	46.1
10	144.7	144.6	147.0
11	42.1	42.2	139.7
12	178.4	178.3	172.4
13	54.3	46.2	123.3
14	115.4	115.8	118.1
15	111.1	111.6	113.4
Glc 1	104.4	104.3	106.9
Glc 2	75.1	75.1	77.3
Glc 3	78.3	78.3	80.5
Glc 4	71.6	71.6	73.6
Glc 5	78.3	78.3	80.4
Glc 6	62.6	62.7	64.8
1''	176.8	176.7	
2''	67.8	18.5	
3''	29.5	58.2	
4''	24.1		
5''	52.6		

Table 3. Inhibitory effect of **1-14** on the proliferation of tumor cell lines, *in vitro*

Compound	[a]ED_{50} (μM)			
	MES-SA	MES-SA/DX5	HCT15	HCT15/CL02
1	> 30.00	> 30.00	> 30.00	> 30.00
2	> 30.00	> 30.00	> 30.00	> 30.00
3	> 30.00	> 30.00	> 30.00	> 30.00
4	> 30.00	> 30.00	> 30.00	> 30.00
5	25.04 ± 0.73	> 30.00	23.92 ± 0.37	> 30.00
6	> 30.00	> 30.00	> 30.00	> 30.00
7	25.02 ± 0.32	29.32 ± 0.43	25.84 ± 0.18	26.81 ± 0.36
8	> 30.00	> 30.00	> 30.00	> 30.00
9	> 30.00	> 30.00	> 30.00	> 30.00
10	> 30.00	> 30.00	> 30.00	> 30.00
11	> 30.00	> 30.00	> 30.00	> 30.00
12	> 30.00	> 30.00	> 30.00	> 30.00
13	1.37 ± 0.34	2.07 ± 0.17	1.52 ± 0.12	2.64 ± 0.34
14	1.98 ± 0.26	3.08 ± 0.18	2.51 ± 0.24	3.48 ± 0.12
Etoposide	0.40 ± 0.12	11.00 ± 0.21	2.00 ± 0.24	1.80 ± 0.31

[a]ED_{50} value of compounds against each cancer cell lines, which was defined as the concentration that caused 50% inhibition of cell proliferation *in vitro*. Each data is expressed as the mean ± S.D. of three distinct experiments. Etoposide was used as a positive reference.

−21.6 (*c* 0.15, CH_3OH; ^{1}H NMR (pyridine-d_5, 800 MHz); ^{13}C NMR (pyridine-d_5, 200 MHz) (Table 1); HRESIMS *m/z* 540.2439 $[M+H]^+$ (calcd for $C_{26}H_{37}NO_{11}$, 540.2436).

Ixerisamine B (2): White amorphous powder; $[\alpha]_D^{20}$ −7.8 (*c* 0.165, CH_3OH); ^{1}H NMR (pyridine-d_5, 800 MHz); ^{13}C NMR (pyridine-d_5, 200 MHz) (Table 1); HRESIMS *m/z* 541.2283 $[M+H]^+$ (calcd. for $C_{24}H_{35}NO_{11}$, 514.2281).

Treatment of 1 with Ammonia. Compound **1** (10 mg) were stirred in 1 mL of 5% NH_4OH at room temperature for 12 h. The reaction mixture was purified with RP-18 column chromatography (5-20% MeOH) to give 4.2 mg of **3**, 1.2 mg of L-proline and 1.0 mg of **15**.

15: White amorphous powder; $[\alpha]_D^{20}$ +47.0 (*c* 0.16, H_2O); ESIMS *m/z* 464.2 $[M+Na]^+$; ^{1}H-NMR (300 MHz, pyridine-d_5) δ 5.92 (1H, s, H-15a), 5.56 (1H, s, H-15b), 5.18 (1H, s, H-14a), 5.07 (1H, d, *J* = 7.8 Hz, Glc H-1'), 5.03 (1H, s, H-14b), 4.98 (1H, m, H-6), 4.86 (1H, m, H-3), 4.59 (1H, m, Glc H-6'a), 4.41 (1H, m, H-8), 4.35 (1H, m, Glc H-6'b), 4.35 (1H, m, Glc H-2'), 4.01 (1H, m, Glc H-5'), 3.49 (1H, m, H-11), 3.34 (1H, m, H-13a), 3.15 (1H, m, H-13b), 2.86 (1H, m, H-1), 2.77 (1H, m, H-5), 2.70 (1H, m, H-7), 2.53 (1H, m, H-9a), 2.42 (1H, m, H-9b), 2.21 (2H, m, H-2).

Cytotoxicity Assessment. The cytotoxicity of compounds against cultured human tumor cell lines was evaluated by the SRB method.[13] The ED_{50} values of compounds were calculated by the nonlinear regression analysis and expressed as the mean ± S.D. of three distinct experiments.

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