

Dentatins: Sesquiterpene Glucosides from *Ixeris dentata*

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Three new sesquiterpene lactone glucosides named dentatins A, B and C were isolated from *Ixeris dentata* and the structures were elucidated as 3 β , 8 β -dihydroxy-(1 α , 5 α)-guaian-10(14)-ene-6 α , 12-olide-3-O- β -D-glucopyranoside (1), 3 β , 8 β -dihydroxy-(1 α , 5 α)-guaian-4(15), 10(14)-diene-6 α , 12-olide-3-O- β -D-glucopyranoside (2) and 3 β , 9 β -dihydroxy-(1 α , 5 α)-guaian-4(15), 10(14)-diene-6 α , 12-olide-3-O- β -D-glucopyranoside (3), respectively, on the basis of spectral evidence.

Key words: *Ixeris dentata*, Compositae, Guaiane type sesquiterpenes, Dentatins

INTRODUCTION

The whole plants of *Ixeris dentata* (Compositae) have been used for treatment of diabetes and gastroenteric troubles in folk medicine (Choi *et al.*, 1990) and the young shoots are edible as a bitter appetizing vegetable in Korea.

In the previous paper (Chung *et al.*, 1994), we reported the isolation of a sesquiterpene lactone as a bitter principle from the ethylacetate-soluble fraction of the methanol extracts from the whole plants. A further study on the butanol-soluble fraction has now led to the isolation of three new bitter sesquiterpene glucosides named dentatins, A (1), B (2) and C (3).

MATERIALS AND METHODS

General Experimental Procedures

Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. IR measurements were recorded in KBr pellets on a JASCO FT/IR-5300 Spectrometer. FAB-MS and EI-MS spectra were obtained on a Finnigan MAT 90 and Hewlett Packard Model 5985B GC/MS System, respectively. ¹H (500 MHz) and ¹³C (125 MHz) NMR spectra were taken in pyridine-d₅ with TMS as internal standard on a BRUKER AMX-500 Super-conducting FT-NMR Spectrometer and chemical shifts are reported in δ (ppm) and coupling con-

stants are in Hz.

Plant Material

The fresh whole plants of *I. dentata* were collected in the field of Yeu Ju, Kyung Ki Do. A voucher specimen deposited at Kwangnung Arboretum, Chungbu Forestry Experiment Station.

Isolation of Dentatins A, B and C

The MeOH extract from the air-dried whole plants (7 kg) was dissolved in water and extracted with CHCl₃ and then with EtOAc. The aq. layer was partitioned with BuOH and the BuOH layer was concentrated before chromatography on a silica gel column. The column was eluted with CHCl₃ and subsequently with a gradient of CHCl₃-MeOH to afford 7 fractions. Fractions 3-5 which showed bitter taste, were rechromatographed separately to give dentatins A (1, 80 mg), B (2, 50 mg) and C (3, 45 mg), respectively.

Dentatin A (1) [3 β , 8 β -dihydroxy-(1 α , 5 α)-guaian-10(14)-ene-6 α , 12-olide-3-O- β -D-glucopyranoside]: Colorless powder from MeOH; mp 208°; Liebermann-Burchard, +; Molisch, +; IR ν_{\max}^{KBr} cm⁻¹: 3450 (OH), 1740 (γ -lactone), 1100-1000 (glycoside); EI-MS(30 eV) *m/z* (rel. int., %): 428 [M]⁺ (0.7), 266 [M-C₆H₁₀O₅]⁺ (5.7); ¹H and ¹³C NMR, see Table I and III.

Dentatin B (2) [3 β , 8 β -dihydroxy-(1 α , 5 α)-guaian-4(15), 10(14)-diene-6 α , 12-olide-3-O- β -D-glucopyranoside]: Colorless powder from MeOH; mp 210°; Lie-

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Table I. ¹H NMR chemical shifts of dentatins (pyridine-d₅)

H	1	2	3
1 _α	2.73(dt)	2.86(dt)	3.63(ddd)
2 _α	2.44(ddd)	2.46(ddd)	2.43(ddd)
2 _β	2.12(ddd)	2.13(ddd)	2.15(ddd)
3 _α	3.86(dt)	4.82(dd)	4.86(t)
4 _α	2.25(tq)		
5 _α	1.77(dt)	2.73(t)	2.92(t)
6 _β	4.46(t)	4.88(t)	4.18(t)
7 _α	2.05(ddd)	2.21(dt)	2.49(ddd)
8 _α	4.12(dt)	4.18(dt)	2.34(dt)
8 _β			1.52(dt)
9 _α	2.77(dd)	2.67(dd)	4.80(dd)
9 _β	2.30(dd)	2.52(dd)	
11 _α	3.21(qnt)	3.26(dq)	2.35(dq)
13	1.24(d)	1.28(d)	1.23(d)
14 _A	5.12(br s)	5.18(br s)	5.13(br s)
14 _B	5.04(br s)	5.0(br s)	5.10(br s)
15 _A	1.37(d)	5.93(br s)	5.94(br s)
15 _B		5.59(br s)	5.51(br s)
1'	4.88(d)	5.09(d)	5.04(d)
2'	4.06(dd)	4.06(dd)	4.10(dd)
3'	4.27(dd)	4.27(dd)	4.35(dd)
4'	4.22(t)	4.22(t)	4.25(t)
5'	3.97(ddd)	3.97(ddd)	4.06(ddd)
6' _A	4.55(dd)	4.55(dd)	4.55(dd)
6' _B	4.38(dd)	4.38(dd)	4.39(dd)

The assignments were made by a combination of COSY and HETCOR.

J[Hz]: compound 1: 1_α2_α=1_α5_α=9.5; 1_α2_β=2.9; 2_α2_β=12.8; 2_α3_α=7.0; 2_β3_α=5_α6_β=6_β7_α=9.9; 3_α4_α=7.7; 4_α5_α=8.1; 4_α15=6.6; 7_α8_α=2.6; 7_α11_α=11_α 13=7.3; 8_α9_α=8_α9_β=4.8; 9_α9_β=14.2; 3_α2'=14_A9_α=14_B9_α=ca 1.0; sugar: 1'2'=6.9; 2'3'=7.3; 3'4'=8.6; 4'5'=8.7; 5'6'_A=2.6; 5'6'_B=5.6; 6'_A6'_B=12.0; compound 2: 1_α2_α=1_α5_α=9.5; 1_α2_β=4.8; 2_α2_β=9_α9_β=14.3; 2_α3_α=7_α11_α=7.7; 2_β3_α=5_α6_β=6_β7_α=9.9; 7_α8_α=2.9; 8_α9_α=3.3; 8_α9_β=4.0; 11_α13=6.9; 15_A3_α=15_A5_α=15_B3_α=15_B5_α=6_A1'=ca 1.0; sugar: 1'2'=7.8; 2'3'=7.3; 3'4'=8.6; 4'5'=8.7; 5'6'_A=2.6; 5'6'_B=5.6; 6'_A6'_B=12.0; compound 3: 1_α2_α=6.8; 1_α5_α=5_α6_β=9.4; 1_α2_β=7_α8_α=2.6; 2_α2_β=13.7; 2_α3_α=2_β3_α=9.5; 6_β7_α=9.1; 7_α8_β=11.5; 7_α11_α=7.7; 8_α8_β=11.7; 8_α9_α=3.3; 8_β9_α=9.7; 11_α13=7.0; 5_α7_α=6_β8_β=6_β11_α=7_α13=15_A3_α=15_A5_α=15_A1'=15_A6'_B=15_B3_α=15_B5_α=15_B6_β=ca 1.0; sugar: 1'2'=7.8; 2'3'=8.3; 3'4'=7.8; 4'5'=8.0; 5'6'_A=2.9; 5'6'_B=5.6; 6'_A6'_B=11.6.

bermann-Burchard, +; Molisch, +; [α]_D²² +25° (c=0.28, MeOH); IR ν_{max}^{KBr} cm⁻¹: 3450 (OH), 1740 (γ-lactone), 1100-1000 (glycoside); EI-MS (30 eV) m/z (rel. int., %): 426 [M]⁺ (0.8), 264 [M-C₆H₁₀O₅]⁺ (4.7); ¹H and ¹³C NMR, see Table I and III.

Dentatin C (3) [3β, 9β-dihydroxy-(1α, 5α)-guaian-4(15), 10(14)-diene-6α, 12-olide-3-O-β-D-glucopyranoside]: Colorless powder from MeOH; mp 212°; Liebermann-Burchard, +; Molisch, +; [α]_D²² -40° (c=0.09, MeOH); IR ν_{max}^{KBr} cm⁻¹: 3385 (OH), 1736 (γ-lactone), 1100-1000 (glycoside); Positive FAB-MS m/z (rel.

Table II. ¹H NMR chemical shifts of dentalactones (pyridine-d₅)

H	1a	2a	3a
1 _α	2.70(m)	2.70(dd)	3.66(ddd)
2 _α	2.45(ddd)	2.46(m)	2.46(m)
2 _β	2.19(ddd)	2.20(m)	2.09(m)
3 _α	3.93(dt)	4.82(m)	4.83(m)
4 _α	2.30(m)		
5 _α	1.84(dt)	2.82(m)	2.98(t)
6 _β	4.63(t)	4.82(m)	4.14(t)
7 _α	2.06(m)	2.20(m)	2.56(m)
8 _α	4.16(dt)	4.19(dt)	2.31(m)
8 _β			1.44(dt)
9 _α	2.81(m)	2.82(m)	4.80(m)
9 _β	2.29(m)	2.46(m)	
11 _α	3.19(dq)	3.27(dq)	2.36(m)
13	1.26(d)	1.20(d)	1.20(d)
14 _A	5.23(br s)	5.25(br s)	5.20(br s)
14 _B	5.12(br s)	5.08(br s)	5.10(br s)
15 _A	1.42(d)	5.68(br s)	5.71(br s)
15 _B		5.58(br s)	5.57(br s)

Table III. ¹³C NMR chemical shifts of dentatins (pyridine-d₅)

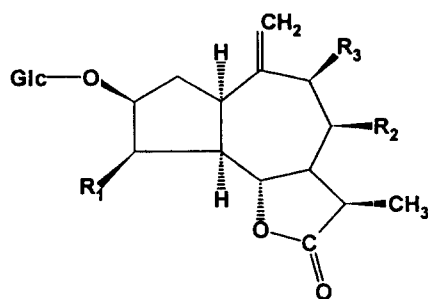
H	1	2	3
1	43.1(CH)*	44.5(CH)*	35.6(CH)*
2	38.4(CH ₂)	38.3(CH ₂)	37.2(CH ₂)
3	87.3(CH)	80.6(CH)	80.6(CH)
4	45.4(CH)	143.8(C)	154.4(C)
5	51.5(CH)	50.6(CH)	48.9(CH)
6	80.6(CH)	78.6(CH)	83.9(CH)
7	56.2(CH)	54.0(CH)	44.4(CH)
8	63.6(CH)	64.4(CH)	40.0(CH ₂)
9	45.2(CH ₂)	44.4(CH ₂)	72.8(CH)
10	144.2(C)	144.6(C)	150.6(C)
11	36.9(CH)	36.7(CH)	42.2(CH)
12	179.0(C)	179.0(C)	178.9(C)
13	13.4(CH ₃)	13.4(CH ₃)	12.8(CH ₃)
14	115.4(CH ₂)	115.5(CH ₂)	111.7(CH ₂)
15	18.6(CH ₃)	115.4(CH ₂)	110.6(CH ₂)
1'	105.8(CH)	104.6(CH)	105.0(CH)
2'	75.4(CH)	75.4(CH)	75.0(CH)
3'	78.6(CH)	78.6(CH)	78.9(CH)
4'	71.6(CH)	71.7(CH)	70.9(CH)
5'	78.5(CH)	78.5(CH)	77.8(CH)
6'	62.8(CH)	62.8(CH)	62.2(CH)

*Sorted by APT experiment.

int., %): 427 [M+H]⁺ (54.1), 265 [M+H-C₆H₁₀O₅]⁺ (79.3); Negative FAB-MS m/z (rel. int., %): 425 [M-H]⁻ (100.0), 263 [M-H-C₆H₁₀O₅]⁻ (10.1); ¹H and ¹³C NMR, see Table I and III.

Enzymatic Hydrolysis of Dentatins A, B and C

Each compound (20 mg) in DMSO/H₂O (2 ml) was treated with β-glucosidase (Sigma Chemical Co., EC



	R ₁	R ₂	R ₃
1	CH ₃	OH	H
2	CH ₂ =	OH	H
3	CH ₂ =	H	OH

3.2.1.21, 20 mg) for 12 hrs at 38° with stirring, then the reaction mixture was extracted with CHCl₃ to give genins named dentalactones A (**1a**) (formerly dentalactone, previously isolated from this plant, Chung *et al.*, 1994), B (**2a**) and C (**3a**) from **1**, **2** and **3**, respectively.

Dentalactone A (1a): White amorphous powder from MeOH; mp 198°; Liebermann-Burchard, +, IR ν_{\max}^{KBr} cm⁻¹: 3430 (OH), 1736 (γ -lactone); EI-MS (30 eV) m/z (rel. int., %): 266 [M]⁺ (7.5), 248 [M-H₂O]⁺ (10.6), 230 [M-2H₂O]⁺ (11.8); ¹H NMR, see Table II.

Dentalactone B (2a): Colorless amorphous powder from MeOH; mp 208°; Liebermann-Burchard, +, IR ν_{\max}^{KBr} cm⁻¹: 3430 (OH), 1740 (γ -lactone); EI-MS (70 eV) m/z (rel. int., %): 264 [M]⁺ (2.3), 246 [M-H₂O]⁺ (4.2), 228 [M-2H₂O]⁺ (2.8); ¹H NMR, see Table II.

Dentalactone C (3a): Colorless amorphous powder from MeOH; mp 226°; Liebermann-Burchard, +, IR ν_{\max}^{KBr} cm⁻¹: 3400 (OH), 1740 (γ -lactone); EI-MS (30 eV) m/z (rel. int., %): 264 [M]⁺ (79.3), 246 [M-H₂O]⁺ (47.0); ¹H NMR, see Table II.

Each aq. layer was evaporated under reduced pressure. The residue was found to contain D-glucose by TLC (precoated cellulose, pyridine-EtOAc-HOAc-H₂O=36:36:7:21) by comparison with an authentic sugar.

RESULTS AND DISCUSSION

All dentatins gave a positive reaction in Molisch test besides Liebermann Burchard test and showed the presence of a γ -lactone (around 1740 cm⁻¹) and a glycoside bond (1100-1000 cm⁻¹) in IR spectra. Enzymatic hydrolysis of dentatins afforded glucose as a sugar moiety and the corresponding genins (dentalactones A, B and C).

Dentalactone A (**1a**), the aglucone of dentatin A (**1**), was found to be identical with 3 β , 8 β -dihydroxy-(1 α ,

5 α)-guaian-10(14)-ene-6 α , 12-olide by direct comparison (mmp, co-TLC, EI-MS and ¹H NMR) with an authentic sample of dentalactone (Chung *et al.*, 1994).

The mass spectrum of dentatin A (**1**) exhibited a molecular ion peak at m/z 428 and a fragment ion peak at m/z 266 corresponding to the aglucone, indicating the presence of one mole of sugar.

Comparison of the ¹³C NMR signals of **1** with those of **1a** showed significant glycosidation shift (+9.3 ppm) of the signal for C-3, indicating that glucose was linked to the 3 β -OH group. Considering the coupling constant of the anomeric proton ($J=6.9$ Hz) and the chemical shift of the anomeric carbon (δ 105.8), C-1 atom of glucose was assigned as β -configuration.

On the basis of the above evidence, the structure of dentatin A (**1**) was elucidated to be 3-O- β -D-glucopyranosyldentalactone A.

The mass spectrum of dentalactone B (**2a**) exhibited a molecular ion peak at m/z 264, in accord with the formula C₁₅H₂₀O₄, as well as fragment ions at m/z 246 [M-H₂O]⁺ and 228 [M-2H₂O]⁺, indicating the presence of two hydroxyl groups. And the mass spectrum of dentatin B (**2**) showed a molecular ion peak at m/z 426 and a fragment ion peak at m/z 264 corresponding to the aglucone, showing the presence of one mole of sugar.

In comparison of the ¹H and ¹³C NMR of **2** and **2a** with those of **1** and **1a**, all of the signals of both compounds appeared at almost the same positions with the exception of appearance of two broad singlets at δ 5.93 and 5.59 and δ 5.68 and 5.58, respectively, and disappearance of signals at δ 1.37 and 1.42 for the methyl protons at C-15 and δ 2.25 and 2.30 for the methine proton at C-4 and shift of the carbon resonances at δ 45.4 and 18.6 to δ 143.8 and 115.4, respectively, indicating that an additional exocyclic methylene group was clearly allocated to C-4 of dentatin B. Therefore, the structure of dentatin B (**2**) was elucidated to be 3 β , 8 β -dihydroxy-(1 α , 5 α)-guaian-4(15), 10(14)-diene-6 α , 12-olide-3-O- β -D-glucopyranoside.

Dentalactone C (**3a**) having a molecular formula C₁₅H₂₀O₄ (M⁺, m/z 264) showed the presence of a methyl group (δ 1.20), two exocyclic methylene groups (δ 5.10 and 5.20 and δ 5.57 and 5.71) and two hydroxy groups (δ 4.80 and 4.83) in ¹H NMR spectrum. Positive ion FABMS of dentatin C (**3**), in agreement with its molecular formula C₂₁H₃₀O₉, gave rise to a peak at m/z 427 corresponding to the quasi-molecular ion [M+H]⁺ and a fragment ion peak at m/z 265 corresponding to the aglucone, indicating the presence of one mole of glucose.

The ¹³C NMR spectrum displayed 21 carbon resonances. Lactone carbonyl resonances were located at δ 83.9 and 178.9 and two resonances for oxygen bearing carbons were observed at δ 72.8 and 80.6, two exocy-

clic methylene resonances at δ 111.7 and 150.6 and δ 110.6 and 154.4 and glucose carbon resonances at δ 105.0, 75.0, 78.9, 70.9, 77.8, 62.2, respectively. Judging from the APT spectrum, it is clear that the remaining carbon resonances are due to one methyl, two methylenes and four methines. The splitting pattern of the oxymethine proton signal at δ 4.18 (1H, t, $J_{5,6}$ = 9.4 Hz, $J_{6,7}$ = 9.1 Hz) correlated with the ^{13}C resonance at δ 83.9 and was assignable to the lactonic proton at C-6, indicating the *trans*-diaxial disposition of the protons at C-5 (α), C-6 (β), C-7 (α). This suggested that dentatin C (**3**) was also a guaianolide-type sesquiterpene lactone glucoside.

2D COSY experiments allowed the assignment of the double triplet at δ 4.86 and the double doublet at δ 4.80 to the oxymethine protons at C-3 and C-9, respectively, and the coupling constant between H-2 and H-3 was 9.5 Hz and that between H-8 and H-9 was 3.3 Hz and 9.7 Hz, supporting a β -orientation of the two hydroxyl groups.

The doublet at δ 1.23 was assignable to the methyl protons at C-13 and the coupling constant between H-11 and H-13 was 7.0 Hz, suggesting that the methyl group was also β -oriented. Two broad singlets at δ 5.51 and 5.94 clearly represented methylene protons at C-

15, which exhibited long range coupling with H-3 and H-5. Accordingly, remaining two broad singlets at δ 5.10 and 5.13 were assigned to H-14.

Appearance of the chemical shift of C-3 in rather lower field (δ 80.6) indicated the attachment of glucose to the C-3 position. The β -configuration of glucosidic linkage was suggested not only from the coupling constant of the anomeric proton (J = 7.8 Hz) but also from the chemical shift of the anomeric carbon (δ 105.0).

Consequently, the structure of **3** was established as 3β , 9β -dihydroxy-(1α , 5α)-guaian-4(15), 10(14)-diene- 6α , 12-olide-3-O- β -D-glucopyranoside. To the best of our knowledge the isolation of dentatins A, B and C from a natural source seems not to have been reported previously.

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