[Chem. Pharm. Bull. 34(9)3769—3773(1986)]

Sesquiterpene Glycosides from *Youngia denticulata* (HOUTT.) KITAM.

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(Received March 15, 1986)

Four new guaiane-type sesquiterpene glycosides have been isolated from *Youngia denticulata* (HOUTT.) KITAM. (Compositae), in addition to two known compounds, picriside C and crepidiaside A. Their structures were elucidated on the basis of spectral data and some chemical transformations.

Keywords——*Youngia denticulata*; Compositae; sesquiterpene glycoside; sesquiterpene lactone; picriside C; crepidiaside A; youngiaside A; youngiaside B; youngiaside C; youngiaside D

In a continuation of our studies on sesquiterpene glycosides from Compositae plants, we now report the isolation of several closely related sesquiterpene lactone glycosides, youngiasides A—D, in addition to two known glycosides, picriside C and crepidiaside A, from the methanol extract of *Youngia denticulata* (HOUTT.) KITAM. (syn. *Paraixeris denticulata* NAKAI). The structures of these compounds were elucidated on the basis of spectroscopic studies and some chemical transformations.

Picriside C (I): The proton nuclear magnetic resonance (^{1}H-NMR) spectrum exhibited two vinyl methyl signals at δ 1.36 (3H, br s) and 1.96 (3H, br s), and two doublet signals at δ 5.53 (1H, d, J=3.1 Hz) and 6.36 (1H, d, J=3.6 Hz), which are characteristic of exocyclic methylene protons of the α-methylene-γ-lactone grouping common in sesquiterpene lactones. These spectral data were identical to those that we previously reported for picriside C.[1)]

Crepidiaside A (II): The ^{1}H-NMR spectrum exhibited a vinyl methyl signal at δ 2.46 (3H, br s), two exomethylene signals at δ 5.37 (1H, d, J=3.1 Hz) and 6.17 (1H, d, J=3.2 Hz) and an olefinic proton signal at δ 6.91 (1H, br s). These spectral data were identical to those that we previously reported for crepidiaside A.[2)]

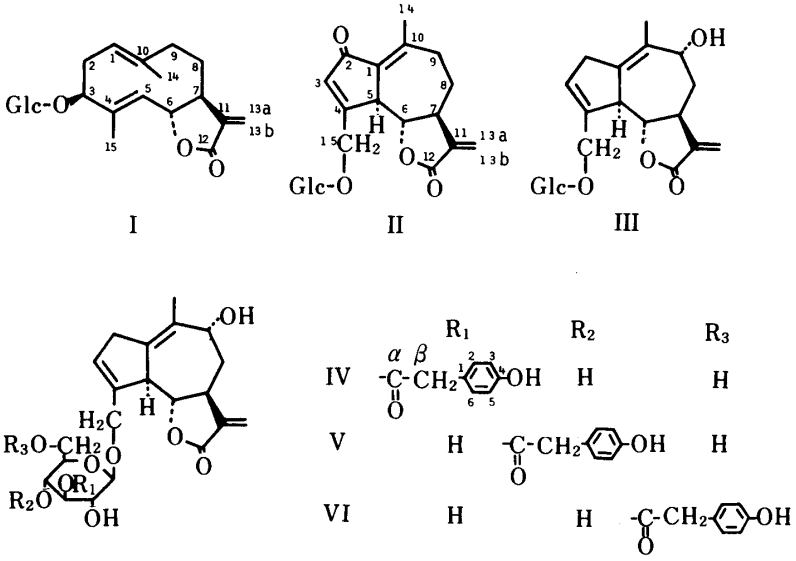

Chart 1

Youngiaside A (III): $C_{21}H_{28}O_9 \cdot H_2O$, $[\alpha]_D$ $-6.7°$. The infrared (IR) spectrum suggested the presence of hydroxyl groups (3430 cm^{-1}) and a γ-lactone group (1760 cm^{-1}). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, twenty-one signals were observed including six signals due to a glucopyranosyl moiety.

Acid hydrolysis of III afforded glucose as the sugar moiety and enzymatic hydrolysis with crude hesperidinase afforded an unstable aglycone (IIIa). The mass spectrum (MS) of IIIa showed a molecular ion peak at m/z 262 in agreement with the molecular formula $C_{15}H_{18}O_4$.

The ^{1}H-NMR spectrum of IIIa exhibited a vinyl methyl signal at δ 1.72 (3H, d, J=1.5 Hz) and two doublet signals at δ 5.37 (1H, d, J=3.1 Hz) and 6.15 (1H, d, J=3.2 Hz) characteristic of the H-13 exocyclic methylene protons. A lactonic proton signal was observed at δ 4.32 as a triplet having a coupling constant of 10 Hz, so H-5, H-6 and H-7 are in anti-diaxial relationships.[3] Irradiation at δ 4.32 (H-6) affected two methine signals at δ 3.80 (1H, d, J=10 Hz) and 3.84 (1H, m), and the latter was also affected by irradiation at the exomethylene signals (H-13). We assigned two methine signals at δ 3.80 and 3.84 to H-5 and H-7, respectively. These two signals were shifted markedly downfield in comparison with those of other similar sesquiterpenes.[4] This result led us to assume that IIIa has an α-hydroxyl group at C-9. Irradiation at δ 3.80 (H-5) affected a methine signal at δ 4.32 (H-6) and AB type methylene signals at δ 4.68 (1H, br d, J=15 Hz) and 4.93 (1H, br d, J=15 Hz), and the latter

TABLE I. ^{13}C-NMR Chemical Shifts and Coupling Constants

Carbon No.	I	II	III	IIIa	IV	V	VI
Aglycone moiety							
1	125.2	131.8	136.2[d]	136.7[f]	136.1[g]	136.0[h]	136.3[i]
2	33.5	194.9	38.5	38.5	38.5	38.5	38.6
3	83.3	134.6	128.1	124.8	128.5	128.1	128.1
4	140.8[a]	169.1	141.8	147.0	141.7	141.7	142.0
5	126.9	50.1	52.3	52.7	52.1	52.2	52.4
6	81.2	84.1	85.4	85.4	85.5	85.4	85.5
7	50.1	52.5	44.0	44.3	44.0	44.0	44.2
8	28.4	24.4	32.8	32.9	32.8	32.8	33.0
9	41.1	37.2	71.5	71.5	71.5	71.4	71.6
10	137.7	152.5	134.8[d]	134.7[f]	135.0[g]	134.9[h]	134.9[i]
11	141.8[a]	139.7	141.2	141.5	141.3	141.2	141.5
12	170.1	169.1	169.9	169.5	169.7	169.7	169.6
13	119.5	118.2	116.9	116.4	116.6	116.6	116.5
14	16.3	21.5	22.4	22.4	22.4	22.3	22.4
15	12.3	68.7	68.1	61.7	68.1	68.2	68.1
Sugar moiety							
1	102.5	104.2	103.1 (155 Hz)		102.8	103.1	103.2
2	75.0	75.1	74.9		72.9	75.1	75.0[j]
3	78.2[b]	78.4[c]	78.2[e]		79.5	75.6	78.3
4	71.7	71.6	71.5		69.4	72.7	71.6
5	78.1[b]	78.2[c]	77.7[e]		77.8	75.6	75.1[j]
6	62.8	62.7	62.6		62.1	61.9	65.0
p-Hydroxyphenylacetic acid moiety							
α					172.0	171.6	172.2
β					40.9	40.7	40.5
1					125.4	125.1	125.4
2, 6					130.8	130.8	131.0
3, 5					116.0	116.1	116.2
4					157.6	157.7	157.8

Run at 22.5 MHz in pyridine-d_5 solution. *a*—*j*) Assignments may be interchanged in each column.

was also coupled with an olefinic proton signal at δ 6.18 (1H, br s). Thus, we supposed that IIIa has a hydroxymethyl group at C-4 and a double bond between C-3 and C-4, and consequently a vinyl methyl group at C-10. Furthermore, irradiation at a vinyl methyl signal at δ 1.72 (H-14) affected a methylene signal at δ 3.04 (2H, br s), a methine signal at δ 3.80 (H-5) and a carbinol proton signal at δ 4.47 (1H, dd, J=6, 1.5 Hz), so we could assign the methylene signal at δ 3.04 to H-2 and the carbinol proton signal at δ 4.47 to H-9.

In the ^{13}C-NMR spectrum of III, C-7 exhibited an upfield shift of 8.5 ppm, and C-9 and C-8 exhibited downfield shifts of 34.3 and 8.4 ppm, in comparison with those of II (Table I). This result also suggested that the O-function exists at C-9.

If the assumption is made that the absolute configuration of the C-7 side chain is as shown (as in all other known sesquiterpene lactones having authenticated stereochemistry), the structure of the aglycone can be concluded to be IIIa. A comparison of the ^{13}C-NMR spectrum of III with that of IIIa exhibited glycosidation shifts at C-15 (Δ+6.4 ppm) and C-4 (Δ−5.2 ppm).[5] Thus, we decided that youngiaside A is a sesquiterpene glucoside having the structure III. The stereochemistry of the anomeric center was deduced to be β from the $J_{C_1-H_1}$ coupling constant (155 Hz).[6]

Youngiasides B (IV), C (V) and D (VI) had the same molecular formula $C_{29}H_{34}O_{11}$ and

TABLE II. ^{1}H-NMR Chemical Shifts and Coupling Constants

Proton No.	I	II	III	IIIa
2			2.92 (2H, br s)	3.04 (2H, br s)
3		6.91 (1H, br s)	6.14[a]	6.18 (1H, br s)
5				3.80 (1H, d, J=10 Hz)
6				4.32 (1H, t, J=10 Hz)
7				3.84 (1H, m)
8α				2.47 (1H, dd, J=6, 2 Hz)
8β				2.31 (1H, m)
9				4.47 (1H, dd, J=6, 1.5 Hz)
13a	5.53 (1H, d, J=3.1 Hz)	5.37 (1H, d, J=3.1 Hz)	5.32 (1H, br s)	5.37 (1H, d, J=3.1 Hz)
13b	6.36 (1H, d, J=3.6 Hz)	6.17 (1H, d, J=3.2 Hz)	6.14[a]	6.15 (1H, d, J=3.2 Hz)
14	1.36 (3H, br s)	2.46 (3H, br s)	1.68 (3H, br s)	1.72 (3H, d, J=1.5 Hz)
15	1.96 (3H, br s)			4.68 (1H, br d, J=15 Hz)
				4.93 (1H, br d, J=15 Hz)

Proton No.	IV	V	VI
Aglycone moiety			
2	2.94 (2H, br s)	2.94 (2H, br s)	2.92 (2H, br s)
3	6.12[a]	6.12[a]	6.14[a]
13a	5.32 (1H, br s)	5.32 (1H, br s)	5.30 (1H, br s)
13b	6.12[a]	6.12[a]	6.14[a]
14	1.70 (3H, br s)	1.67 (3H, br s)	1.67 (3H, br s)
Sugar moiety			
3	5.93 (1H, t, J=9.5 Hz)		
4		5.72 (1H, t, J=9.5 Hz)	
p-Hydroxyphenylacetic acid moiety			
β	3.78 (2H, br s)	3.78 (2H, br s)	3.80 (2H, br s)
2, 6	7.34 (2H, d, J=8 Hz)	7.36 (2H, d, J=8 Hz)	7.37 (2H, d, J=8 Hz)
3, 5	7.08 (2H, d, J=8 Hz)	7.15 (2H, d, J=8 Hz)	7.15 (2H, d, J=8 Hz)

Run at 89.55 MHz in pyridine-d_5 solution. *a*) Overlapped.

exhibited optical rotations of $[\alpha]_D$ −11.5°, −8.8° and −7.5°, respectively. They each had an absorption maximum at 277 nm in the ultraviolet (UV) spectrum. The ^{1}H-NMR spectra of these compounds were quite similar to that of III, except for the additional presence of A_2B_2 type signals characteristic of the *p*-hydroxyphenylacetic acid moiety (Table II).

Acid hydrolysis of IV, V and VI afforded glucose as the sugar moiety. Treatment of these compounds with acetyl chloride–methanol afforded *p*-hydroxyphenylacetic acid methyl ester and treatment with 2% sodium hydroxide afforded compound III; the products were identified by high-pressure liquid chromatography (HPLC). A comparison of the glucosyl signals in the ^{13}C-NMR spectra with those of III showed acylation shifts (upfield shifts of 2.0 and 2.1 ppm at C-2 and C-4, respectively, and a downfield shift of 1.3 ppm at C-3 in IV; 2.6 and 2.1 ppm at C-3 and C-5, respectively, and 1.2 ppm at C-4 in V; 2.6 ppm at C-5 and a downfield shift of 2.4 ppm at C-6 in VI). These results suggested that *p*-hydroxyphenylacetic acid was esterified at C-3 (IV), C-4 (V) and C-6 (VI), so we concluded that youngiasides B, C and D had the structures IV, V and VI, respectively.

Experimental

Melting points were determined on a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. IR spectra were taken on a JASCO J-202 infrared spectrophotometer and UV spectra on a Shimadzu UV-360 recording spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX 90Q spectrometer (at 89.55 and 22.5 MHz, respectively). Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Gas chromatography (GC) was run on a Hitachi K 53 gas chromatograph. HPLC was run on a Kyowa Seimitsu model K 880 instrument.

Isolation——Air-dried whole plants (10.7 kg) of *Y. denticulata* collected in autumn 1984, in Shizuoka, Japan, were extracted twice with methanol under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with ether and *n*-butanol to give gums, 200 and 120 g, respectively. The *n*-butanol extract was chromatographed repeatedly on a silica gel column, mainly with a chloroform–methanol system so as to give the following sesquiterpene glycosides.

Picriside C (I)——Amorphous powder (60 mg). IR ν_{max}^{KBr} cm^{-1}: 3430, 1765. ^{1}H- and ^{13}C-NMR: Tables I and II.

Crepidiaside A (II)——Amorphous powder (50 mg). IR ν_{max}^{KBr} cm^{-1}: 3420, 1765, 1680, 1635, 1618. ^{1}H- and ^{13}C-NMR: Tables I and II.

Youngiaside A (III)——Amorphous powder (3 g), $[\alpha]_D^{22}$ −6.7° (c=1.04, MeOH). *Anal.* Calcd for $C_{21}H_{28}O_9 \cdot H_2O$: C, 57.01; H, 6.83. Found: C, 57.13; H, 6.75. IR ν_{max}^{KBr} cm^{-1}: 3430, 1760, 1640, 1260. ^{1}H- and ^{13}C-NMR: Tables I and II.

Youngiaside B (IV)——Amorphous powder (80 mg), $[\alpha]_D^{22}$ −11.5° (c=1.09, MeOH). *Anal.* Calcd for $C_{29}H_{34}O_{11} \cdot 1/2\ H_2O$: C, 61.37; H, 6.22. Found: C, 61.14; H, 6.29. IR ν_{max}^{KBr} cm^{-1}: 3430, 1750, 1620, 1520. UV λ_{max}^{MeOH} nm (log ε): 277 (3.35). ^{1}H- and ^{13}C-NMR: Tables I and II.

Youngiaside C (V)——Amorphous powder (1 g), $[\alpha]_D^{24}$ −8.8° (c=1.02, MeOH). *Anal.* Calcd for $C_{29}H_{34}O_{11} \cdot H_2O$: C, 60.41; H, 6.29. Found: C, 60.44; H, 6.19. IR ν_{max}^{KBr} cm^{-1}: 3430, 1750, 1620, 1520. UV λ_{max}^{MeOH} nm (log ε): 277 (3.23). ^{1}H- and ^{13}C-NMR: Tables I and II.

Youngiaside D (VI)——Amorphous powder (120 mg), $[\alpha]_D^{20}$ −7.5° (c=0.20, MeOH). *Anal.* Calcd for $C_{29}H_{34}O_{11} \cdot H_2O$: C, 60.41; H, 6.29. Found: C, 60.22; H, 6.10. IR ν_{max}^{KBr} cm^{-1}: 3430, 1750, 1620, 1520. UV λ_{max}^{MeOH} nm (log ε): 277 (3.32). ^{1}H- and ^{13}C-NMR: Tables I and II.

Enzymatic Hydrolysis of Youngiaside A (III)——Youngiaside A (750 mg) was dissolved in water (10 ml) and stirred with crude hesperidinase (300 mg) for 2 h at 37 °C. After being diluted with water, the mixture was passed through an Amberlite XAD-2 column, which was washed with water. The methanol eluate was purified by silica gel column chromatography to give the aglycone (IIIa) (35 mg), as an amorphous powder. IR ν_{max}^{KBr} cm^{-1}: 3450, 1765, 1670, 1260. MS *m/z*: 262 (M^+, 1), 244 ($M^+ - H_2O$, 6), 226 (3), 215 (3), 213 (3), 181 (7), 163 (6), 149 (8), 131 (10), 123 (11), 105 (11). ^{1}H- and ^{13}C-NMR: Tables I and II.

Hydrolysis of Youngiasides B (IV), C (V) and D (VI)——Youngiaside B (IV) (*ca.* 0.1 mg) in acetyl chloride–methanol (1 : 20) (1 ml) was refluxed for 20 min. The solution was concentrated to give *p*-hydroxyphenylacetic acid methyl ester. Youngiasides C (V) and D (VI) were treated in a similar manner to give *p*-hydroxyphenylacetic acid methyl ester, which was detected by HPLC. HPLC conditions: column, Develosil ODS-7, 4.6 mm × 25 cm; eluent, H_2O–MeOH (50 : 50); detector, UV 205 nm; flow rate, 1.4 ml/min; t_R, 4.9 min.

Saponification of Youngiasides B (IV), C (V) and D (VI)——A solution of youngiaside B (IV) (*ca.* 0.1 mg) in aqueous 2% NaOH (0.1 ml) was stirred for 1 h at room temperature under a nitrogen atmosphere. The solution was

acidified with diluted HCl and extracted with *n*-butanol. The extract was concentrated to give III. Youngiasides C (V) and D (VI) were each saponified in a similar manner to give III, which was detected by HPLC. HPLC conditions: column, Develosil ODS-7, 4.6 mm × 25 cm; eluent, H_2O–MeOH (50 : 50); detector, UV 205 nm; flow rate, 1.4 ml/min; t_R, 6.1 min.

Acid Hydrolysis of Youngiasides A—D——A solution of a glycoside (*ca.* 0.1 mg) in 10% H_2SO_4 (2 drops) was heated on a boiling water bath for 30 min. The solution was passed through an Amberlite IR-45 column, and concentrated to give a residue, which was reduced with $NaBH_4$ (*ca.* 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and concentrated to dryness. Boric acid was removed by distillation with methanol and the residue was acetylated with acetic anhydride (1 drop) and pyridine (1 drop) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. Glucitol acetate was detected by GC. GC conditions: column, 1.5% OV-17, 3 mm × 1 m; column temperature, 215 °C; carrier gas, N_2; t_R, 9.6 min.

Acknowledgement We thank Dr. M. Uchida for measurement of mass spectra and Mrs. H. Kitamura of our college for elemental analyses.

References

1) K. Nishimura, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi, and S. Fukushima, *Chem. Pharm. Bull.*, **34**, 2518 (1986).
2) S. Adegawa, T. Miyase, A. Ueno, T. Noro, M. Kuroyanagi, and S. Fukushima, *Chem. Pharm. Bull.*, **33**, 4906 (1985).
3) W. Herz, K. Aota, M. Holub, and Z. Samek, *J. Org. Chem.*, **35**, 2611 (1970).
4) F. Bohlmann, W. Kramp, R. K. Gupta, R. M. King, and H. Robinson, *Phytochemistry*, **20**, 2375 (1981).
5) O. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985).
6) K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, **1973**, 1037.