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Sesquiterpene Lactones from *Picris hieracioides* L. var. *japonica* REGEL. I

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Three new sesquiterpene glycosides, picrisides A (III), B (VI) and C (VII), in addition to lactucin (I), 11β,13-dihydrolactucin (II), crepidiaside A (IV) and ixerin F (V), have been isolated from the methanol extract of *Picris hieracioides* L. var. *japonica* REGEL (Compositae). The structures of the new compounds were determined on the basis of chemical and spectral data.

Keywords——*Picris hieracioides* var. *japonica*; Compositae; sesquiterpene glycoside; picriside A; picriside B; picriside C; lactucin; 11β,13-dihydrolactucin; crepidiaside A; ixerin F

In the course of a search for sesquiterpene lactone glycosides in Compositae plants,[1] we have investigated the constituents of *Picris hieracioides* L. var. *japonica* REGEL, and isolated a new guaianolide-type glycoside, picriside A, and two new germacranolide-type glycosides, picrisides B and C, along with lactucin,[2] 11β,13-dihydrolactucin,[3] crepidiaside A[4] and ixerin F.[5]

Lactucin (I). From the infrared (IR) and the proton nuclear magnetic resonance (^{1}H-NMR) spectral data, I was assumed to be lactucin, previously isolated from *Lactuca virosa* L.,[6] and its identity was confirmed by comparing the IR, ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra and melting point with those of an authentic sample.[2]

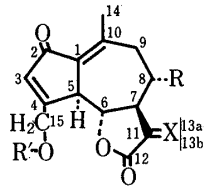
11β,13-Dihydrolactucin (II). The ^{1}H-NMR spectrum of II was similar to that of I. Compound II was shown to be identical with 11β,13-dihydrolactucin, which had been isolated from *Launaea mucronata*, by comparing the IR and ^{1}H-NMR spectra and melting point.[3]

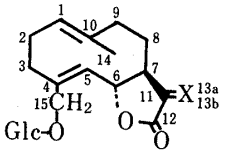
Picriside A (III), $C_{21}H_{26}O_{10}$, mp 248—250 °C, $[\alpha]_D$ −38.0°. The ^{1}H-NMR spectrum was similar to that of I. The ^{13}C-NMR spectrum was also similar to that of I (Table I), but six additional signals were observed, which were assigned to a glucopyranosyl moiety, and the chemical shift of C-15 exhibited a downfield shift of 6.2 ppm compared with that of I. Thus, III was assumed to have a glucopyranosyl group at C-15 of I.[7] Enzymatic hydrolysis of III afforded lactucin (I) as the aglycone, and acid hydrolysis afforded glucose as the sugar moiety. In the ^{1}H-NMR spectrum, the anomeric proton appeared at δ 4.90 (1H, d, J=7 Hz), showing that the glucosidic linkage is β. These results led us to assign the structure III to picriside A.

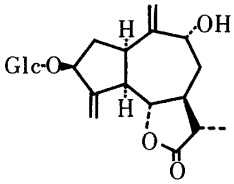
Crepidiaside A (IV). From ^{1}H-NMR spectral data, IV was assumed to be crepidiaside A, which had been isolated from *Crepidiastrum keiskeanum* NAKAI. The identity of IV was established by direct comparison [thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and ^{1}H-NMR] with an authentic sample.[4]

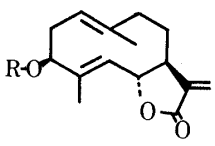
Ixerin F (V). By comparing the ^{1}H- and ^{13}C-NMR spectra, V was shown to be identical with ixerin F, which had been isolated from *Ixeris tamagawaensis* KITAM.[5]

Picriside B (VI), $C_{21}H_{30}O_8 \cdot H_2O$, $[\alpha]_D$ +43.1°. The IR spectrum suggested the presence of hydroxyl groups (3420 cm^{-1}), an α,β-unsaturated γ-lactone (1760 cm^{-1}) and double bonds (1660, 1640 cm^{-1}). The ^{1}H-NMR spectrum exhibited two doublets at δ 6.34 (1H, J=3.3 Hz)









	X	R	R′
I	CH_2	OH	H
II	H, αMe	OH	H
III	CH_2	OH	Glc
IV	CH_2	H	Glc

VI: X=CH_2
VIa: X=H, αMe

V

VII: R=Glc
VIIa: R=H

Chart 1

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

Carbon No.	I	II	III	V	VI	VII
Aglycone moiety						
1	133.2	133.1	133.0	41.9	126.9	125.3
2	194.8	195.1	194.6	37.4	27.8[g]	33.6
3	133.2	133.1	134.7	80.7	35.9	83.3
4	175.0	175.2	169.3[c]	151.3	141.0[h]	140.9[j]
5	49.6[a]	49.6[b]	49.6	49.5	130.1	127.0
6	81.6	81.4	81.6	84.0	80.3	81.2
7	58.2	61.9	58.0	36.6	50.8	50.1
8	67.7	69.3	67.6	40.8	27.1[g]	28.4
9	49.1[a]	49.3[b]	49.0	73.0	41.1	41.2
10	146.4	147.0	146.8	153.6	137.5	137.7
11	138.9	41.9	138.8	45.4	141.1[h]	141.8[j]
12	169.1	177.8	169.0[c]	178.4	170.2	170.1
13	121.9	15.9	122.0	13.3	119.0	119.4
14	21.4	21.5	21.4	111.0[e]	16.2	16.2
15	62.5	62.5	68.7	111.6[e]	67.7	12.3
Sugar moiety						
1			104.1	104.4	105.1	102.7 (153 Hz)
2			75.1	75.3	75.1	75.1
3			78.4[d]	78.5[f]	78.6[i]	78.4[k]
4			71.6	71.9	71.8	71.8
5			78.2[d]	78.1[f]	78.5[i]	78.2[k]
6			62.7	63.0	63.0	62.9

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

and 5.50 (1H, J=3.1 Hz), which are characteristic of exocyclic α-methylene-γ-lactone, and a broad singlet methyl signal at δ 1.36. On the other hand, in the ^{13}C-NMR spectrum, twenty-one signals were observed, including the signals of a glucopyranosyl residue (Table I). Reduction of VI with $NaBH_4$ gave VIa, having a doublet methyl signal at δ 1.21 (J=7 Hz) in its ^{1}H-NMR spectrum. Compound VIa was shown to be identical with ixerin H, which had been isolated from I. *tamagawaensis* KITAM., by comparing the ^{1}H-NMR spectra.[8] The circular dichroism (CD) spectrum of VI showed a negative Cotton effect $[\theta]_{260}$ −919, suggesting that the γ-lactone ring fusion is 6α,7β-*trans*.[9] These results led us to assign the structure VI to picriside B.

Picriside C (VII), $C_{21}H_{30}O_8 \cdot H_2O$, $[\alpha]_D$ +57.1°. The IR spectrum was similar to that of VI, and the ^{1}H-NMR spectrum was also similar to that of VI except for appearance of a

vinylogous methyl signal at δ 1.98. Six signals of a glucopyranosyl residue and fifteen signals which were assignable to the aglycone moiety were observed in the ^{13}C-NMR spectrum (Table I). Enzymatic hydrolysis of VII afforded VIIa as the aglycone. In the ^{1}H-NMR spectrum of VIIa, the signals were assigned on the basis of decoupling experiments. In nuclear Overhauser effect (NOE) experiments, irradiation of the H-3 signal increased the intensity of the H-5 signal, and irradiation of the H-15 methyl signal also produced a positive response at the H-6 signal, so that the C-3 hydroxyl group must be β-oriented. From these results, VIIa was concluded to be 3β-hydroxycostunolide, which had previously been isolated from *Porella japonica*, and the structure of VIIa was finally established by comparing the ^{1}H-NMR spectrum with the reported data.[10] Acid hydrolysis of VII gave glucose as the sugar moiety and the stereochemistry of the anomeric center was deduced from the $J_{C_1-H_1}$ coupling constant (153 Hz).[11] These results led us to conclude the structure of picriside C to be VII.

Experimental

Melting points were determined on Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-140 digital polarimeter. CD spectra were recorded with a JASCO J-20A automatic recording spectropolarimeter. IR spectra were taken on a JASCO A-202 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL FX-90Q (89.55 and 22.5 MHz, respectively) and GX-400 (399.65 MHz) spectrometers. 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 Shimadzu GC-4BPFE gas chromatograph. HPLC was run on a Kyowa Seimitsu model K 880 instrument.

Isolation——Air-dried whole plants (15 kg) of *Picris hieracioides* L. var. *japonica* REGEL were extracted twice with methanol under reflux. The extract was concentrated under refuced pressure and the residue was suspended in water. This suspension was extracted with ether and *n*-butanol, successively. The *n*-butanol-soluble fraction (140 g) was chromatographed on a silica gel column with chloroform–methanol (9 : 1) as an eluent to give compounds I—VII.

Lactucin (I)——Colorless prisms (40 mg). mp 215.0—218.0 °C (methanol). IR ν_{max}^{KBr} cm^{-1}: 3355, 3260, 1760, 1665, 1625, 1610. ^{1}H-NMR (400 MHz) (pyridine-d_5) δ: 2.52 (3H, s, H_3-14), 2.61 (1H, br d, J=13 Hz, H-9α), 2.97 (1H, dd, J=13, 10 Hz, H-9β), 3.27 (1H, br t, J=10 Hz, H-7), 3.67 (1H, t, J=10 Hz, H-6), 3.78 (1H, d, J=10 Hz, H-5), 4.03 (1H, br t, J=10 Hz, H-8), 4.77, 5.35 (each 1H, br d, J=19 Hz, H-15), 6.40 (1H, dd, J=3.1, 1.3 Hz, H-13a), 6.60 (1H, dd, J=3.3, 1.3 Hz, H-13b), 7.01 (1H, br s, H-3). ^{13}C-NMR: Table I.

11β,13-Dihydrolactucin (II)——Colorless crystals (80 mg). mp 94.0—96.0 °C (methanol). IR ν_{max}^{KBr} cm^{-1}: 3520, 3410, 1770, 1680, 1635, 1620. ^{1}H-NMR (90 MHz) ($CDCl_3$) δ: 1.45 (3H, d, J=7 Hz, H_3-13), 2.00—2.42 (2H, m, H-7, H-9β), 2.45 (3H, s, H_3-14), 2.48—2.96 (2H, m, H-9α, H-11), 3.44—3.96 (3H, m, H-5, H-6, H-8), 4.54, 4.88 (each, 1H, br d, J=18 Hz, H-15), 6.44 (1H, br s, H-3). ^{13}C-NMR: Table I.

Picriside A (III)——Colorless crystals (90 mg). mp 248.0—250.0 °C (methanol–ethyl acetate). $[\alpha]_D^{20}$ −38.0° (c=0.71, methanol). *Anal.* Calcd for $C_{21}H_{26}O_9$: C, 57.53; H, 5.98. Found: C, 57.23; H, 6.06. IR ν_{max}^{KBr} cm^{-1}: 3450, 1745, 1692, 1640, 1622. ^{1}H-NMR (90 MHz) (pyridine-d_5) δ: 2.44 (3H, s, H_3-14), 4.90 (1H, d, J=7 Hz, anomeric proton), 4.96, 5.20 (each, 1H, br d, J=18 Hz, H-15), 6.34 (1H, dd, J=3.1, 1.3 Hz, H-13a), 6.56 (1H, dd, J=3.3, 1.3 Hz, H-13b), 6.90 (1H, br s, H-3). ^{13}C-NMR: Table I.

Crepidiaside A (IV)——Amorphous powder (220 mg). ^{1}H-NMR (90 MHz) (pyridine-d_5) δ: 2.45 (3H, s, H_3-14), 4.93 (1H, d, J=7 Hz, anomeric proton), 4.96, 5.22 (each, 1H, br d, J=18 Hz, H-15), 5.36 (1H, d, J=3.1 Hz, H-13a), 6.16 (1H, d, J=3.3 Hz, H-13b), 6.92 (1H, br s, H-3).

Ixerin F (V)——Amorphous powder (50 mg). ^{1}H-NMR (90 MHz) (pyridine-d_5) δ: 1.20 (3H, d, J=7 Hz, H_3-13), 5.08 (2H, br s, H_2-14), 5.45, 5.85 (each 1H, br s, H-15). ^{13}C-NMR: Table I.

Picriside B (VI)——Amorphous powder (130 mg), $[\alpha]_D^{19}$ +43.1° (c=1.01, methanol). *Anal.* Calcd for $C_{21}H_{30}O_8 \cdot H_2O$: C, 58.87; H, 7.53. Found: C, 59.06; H, 7.52. IR ν_{max}^{KBr} cm^{-1}: 3420, 1760, 1660, 1640. ^{1}H-NMR (90 MHz) (pyridine-d_5) δ: 1.36 (3H, br s, H_3-14), 5.50 (1H, d, J=3.1 Hz, H-13a), 6.34 (1H, d, J=3.3 Hz, H-13b). ^{13}C-NMR: Table I. CD (c=4.66×10^{-4}, methanol) [θ] (nm): −919 (260).

Picriside C (VII)——Amorphous powder (130 mg), $[\alpha]_D^{23}$ +57.1° (c=0.28, methanol). *Anal.* Calcd for $C_{21}H_{30}O_8 \cdot H_2O$: C, 58.87; H, 7.53. Found: C, 58.72; H, 7.24. IR ν_{max}^{KBr} cm^{-1}: 3430, 1765, 1660, 1630. ^{1}H-NMR (90 MHz) (pyridine-d_5) δ: 1.37 (3H, br s, H_3-14), 1.98 (3H, br s, H_3-15), 5.55 (1H, d, J=3.1 Hz, H-13a), 6.38 (1H, d, J=3.4 Hz, H-13b). ^{13}C-NMR: Table I. CD (c=1.05×10^{-3}, methanol) [θ] (nm): −5039 (262).

Enzymatic Hydrolysis of Picriside A (III)——Picriside A (*ca.* 1 mg) was dissolved in water (0.2 ml) and the solution was treated with crude hesperidinase (*ca.* 1 mg) for 3 h at 35 °C with stirring. The solution was extracted with ethyl acetate. After concentration of the organic layer, the aglycone was identified as lactucin by HPLC. Conditions: column, YMC-Pack AM-312 6 mm×15 cm; solvent, acetonitrile–water (3 : 7); 1.2 ml/min; detector, UV 258 nm; t_R

3.7 min.

Enzymatic Hydrolysis of Picriside C (VII)——Picriside C (12 mg) was dissolved in water (2 ml) and the solution was treated with crude hesperidinase (4 mg) for 3 h at 35 °C with stirring. The solution was extracted with ethyl acetate, and the extract was purified on a silica gel column to give an aglycone (VIIa) (5 mg). ^{1}H-NMR (400 MHz) ($CDCl_3$) δ: 1.46 (3H, br s, H_3-14), 1.63—1.73 (1H, m, H-8β), 1.74 (3H, br s, H_3-15), 2.05—2.15 (2H, m, H-8α, H-9α), 2.30 (1H, br t, J=10 Hz, H-2β), 2.40—2.50 (2H, m, H-2, H-9β), 2.53 (1H, br t, J=9 Hz, H-7), 4.29 (1H, br dd, J=10, 6 Hz, H-3), 4.62 (1H, t, J=9 Hz, H-6), 4.80 (1H, br d, J=9 Hz, H-5), 4.90 (1H, br d, J=10 Hz, H-1), 5.55 (1H, d, J=2.9 Hz, H-13a), 6.29 (1H, d, J=3.4 Hz, H-13b).

Reduction of Picriside B (VI)——Picriside B (6 mg) was dissolved in methanol (1 ml) and stirred with $NaBH_4$ (5 mg) for 10 min at 0 °C. A small amount of acetic acid and excess water were added, the methanol was evaporated off *in vacuo*, and the residual solution was extracted with *n*-butanol. The *n*-butanol extract was purified by HPLC to give ixerin H (VIa). ^{1}H-NMR (90 MHz) (pyridine-d_5) δ: 1.21 (3H, d, J=7 Hz, H_3-13), 1.35 (3H, br s, H_3-14).

Acid Hydrolysis of Glycosides——A solution of glycoside (*ca.* 1 mg) in 10% sulfuric acid (1 ml) was heated on a boiling water bath for 20 min. The solution was passed through an Amberlite IR-45 column, and the eluate was concentrated to give a residue, which was reduced with sodium borohydride (*ca.* 2 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column, and the eluate was concentrated to dryness. Boric acid was removed by co-distillation with methanol and the residue was acetylated with acetic anhydride and pyridine (each 1 drop) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. Glucitol acetate was detected by GC in the hydrolysate of each glycoside. Conditions: column 2% OV-17, 2 mm × 2 m; column temperature 215 °C; carrier gas, N_2; flow rate, 40 ml/min; t_R 6.2 min.

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