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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), 11$\beta$,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; 11$\beta$,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] 11$\beta$,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]

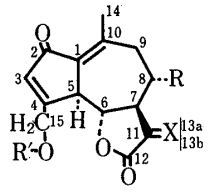
11$\beta$,13-Dihydrolactucin (II). The $^1$H-NMR spectrum of II was similar to that of I. Compound II was shown to be identical with 11$\beta$,13-dihydrolactucin, which had been isolated from *Launaea mucronata*, by comparing the IR and $^1$H-NMR spectra and melting point.[3]

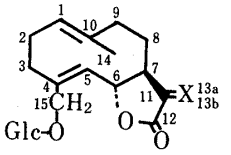
Picriside A (III), $C_{21}H_{26}O_{10}$, mp 248—250 °C, $[\alpha]_D$ −38.0°. The $^1$H-NMR spectrum was similar to that of I. The $^{13}$C-NMR spectrum was also similar to that of I (Table I), but six additional signals were observed, which were assigned to a glucopyranosyl moiety, and the chemical shift 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 $\delta$ 4.90 (1H, d, $J$=7 Hz), showing that the glucosidic linkage is $\beta$. These results led us to assign the structure III to picriside A.

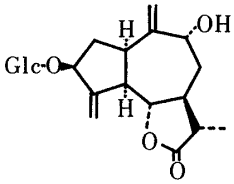
Crepidiaside A (IV). From $^1$H-NMR spectral data, IV was assumed to be crepidiaside A, which had been isolated from *Crepidiastrum keiskeanum* NAKAI. The identity of IV was established by direct comparison [thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and $^1$H-NMR]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

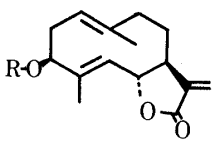
Ixerin F (V). By comparing the $^1$H- and $^{13}$C-NMR spectra, V was shown to be identical with ixerin F, which had been isolated from *Ixeris tamagawaensis* KITAM.[5]

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 $\alpha,\beta$-unsaturated $\gamma$-lactone (1760 cm$^{-1}$) and double bonds (1660, 1640 cm$^{-1}$). The $^1$H-NMR spectrum exhibited two doublets at $\delta$ 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 $\delta$ 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 $\beta$-oriented. From these results, VIIa was concluded to be 3$\beta$-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 $\delta$ (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 $\nu_{max}^{KBr}$ cm$^{-1}$: 3355, 3260, 1760, 1665, 1625, 1610. $^{1}$H-NMR (400 MHz) (pyridine-$d_5$) $\delta$: 2.52 (3H, s, $H_3$-14), 2.61 (1H, br d, $J$=13 Hz, H-9$\alpha$), 2.97 (1H, dd, $J$=13, 10 Hz, H-9$\beta$), 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.

**11$\beta$,13-Dihydrolactucin (II)**——Colorless crystals (80 mg). mp 94.0—96.0 °C (methanol). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3520, 3410, 1770, 1680, 1635, 1620. $^{1}$H-NMR (90 MHz) ($CDCl_3$) $\delta$: 1.45 (3H, d, $J$=7 Hz, $H_3$-13), 2.00—2.42 (2H, m, H-7, H-9$\beta$), 2.45 (3H, s, $H_3$-14), 2.48—2.96 (2H, m, H-9$\alpha$, 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 $\nu_{max}^{KBr}$ cm$^{-1}$: 3450, 1745, 1692, 1640, 1622. $^{1}$H-NMR (90 MHz) (pyridine-$d_5$) $\delta$: 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-$d_5$) $\delta$: 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-$d_5$) $\delta$: 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 $\nu_{max}^{KBr}$ cm$^{-1}$: 3420, 1760, 1660, 1640. $^{1}$H-NMR (90 MHz) (pyridine-$d_5$) $\delta$: 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) [$\theta$] (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 $\nu_{max}^{KBr}$ cm$^{-1}$: 3430, 1765, 1660, 1630. $^{1}$H-NMR (90 MHz) (pyridine-$d_5$) $\delta$: 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) [$\theta$] (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) ($CDCl_3$) $\delta$: 1.46 (3H, br s, $H_3$-14), 1.63—1.73 (1H, m, H-8$\beta$), 1.74 (3H, br s, $H_3$-15), 2.05—2.15 (2H, m, H-8$\alpha$, H-9$\alpha$), 2.30 (1H, br t, $J$=10 Hz, H-2$\beta$), 2.40—2.50 (2H, m, H-2, H-9$\beta$), 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-$d_5$) $\delta$: 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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