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Sesquiterpene Lactones from *Ixeris tamagawaensis* KITAM.

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Three new melampolide-type sesquiterpene lactones have been isolated from the methanol extract of *Ixeris tamagawaensis* KITAM. (Compositae) in addition to luteolin-7-glucoside, and the known sesquiterpene 8-desoxyurospermal A. The structures of the new compounds were elucidated from the spectral data and some chemical transformations.

Keywords——*Ixeris tamagawaensis*; Compositae; sesquiterpene lactone; luteolin-7-glucoside; 8-desoxyurospermal A; ixerin A; ixerin B; ixerin C

In the course of a search for sesquiterpene lactone glycosides in Compositae plants, we have examined the bitter hearb *Ixeris tamagawaensis* KITAM. which is distributed in central Japan. The methanol extract of the whole plant was suspended in water and extracted with ether and *n*-butanol, successively. Polyamide column chromatography of the *n*-butanol extract gave lactones in the H_2O–MeOH (3 : 1) eluate and flavonoid in the acetone eluate. The flavonoid was identified as luteolin-7-glucoside by direct comparison with an authentic sample.

The lactone I has the molecular formula $C_{15}H_{18}O_4$, and its proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed two doublets characteristic of an exocyclic methylene group at δ 6.14 (J=3.3 Hz) and δ 5.48 (J=3.3 Hz). The presence of these olefinic protons indicated an α,β-unsaturated-γ-lactone partial structure. Another prominent signal was the aldehydic proton signal centered at δ 9.42 which was coupled with H-1 (J<1 Hz). The remaining partial structure was evident from the results of spin-decoupling experiments. One olefinic proton at δ 6.54 (br t, J=8.5 Hz) showed long-range coupling with H-14 and another olefinic proton at δ 5.15 (d, J=10.0 Hz) coupled with the triplet signal due to H-6 centered at δ 4.81 (J=10.0 Hz). From these results, I was concluded to be 8-desoxyurospermal A, which had previously been isolated from the root of *Dicoma tomentosa* (Compositae).[1] Thus, the structure of I was finally established by direct comparison [^{1}H-NMR, infrared (IR) spectrum] with an authentic sample.

The lactone II, named ixerin A, has the molecular formula $C_{15}H_{20}O_4$, mp 134—138 °C. Its IR and ^{1}H-NMR spectra were very similar to those of I. It has a 11,13-dihydro-lactone moiety because of the presence of a doublet methyl at δ 1.13 (J=7.0 Hz) instead of two doublets characteristic of an exocyclic methylene group. Thus, we considered that the structure might be II. In the ^{1}H-NMR spectrum, the signal of H-6 appeared as a triplet at δ 5.36 (J=10.0 Hz). This proton was coupled with the H-5 olefinic proton, the latter being responsible for a doublet signal (J=10.0 Hz) centered at δ 5.00. The triplet at δ 6.46 (br J= 8.5 Hz) was ascribed to H-1, which was coupled with an aldehydic proton centered at δ 9.55 (J<1 Hz).

On the other hand, all presently known compounds in the melampolide series have an oxidized C-14, for instance, they are either aldehyde or carboxylic derivatives. It has been pointed out by Fischer that the chemical shift of the aldehydic proton in an α,β-unsaturated medium ring depends on the configuration of the carbon–carbon double bond.[2] Compounds with (Z)-configuration generally exhibit a ^{1}H-NMR signal near 10.0 ppm or above whereas a

value near 9.5 ppm is found in the case of (*E*)-configuration.

Ixerin A showed the aldehydic proton signal at δ 9.55, so that the 1,10-double bond has (*E*)-configuration. In order to determine the configuration of the 4,5-double bond, ixerin A was treated with active MnO_2 to give the dialdehyde (VI). In the ^{1}H-NMR spectrum of VI, the newly produced aldehydic proton appeared as a singlet at δ 10.17, so that the 4,5-double bond was deduced to have (*Z*)-configuration. Futhermore, this was confirmed by nuclear Overhauser effect (NOE) experiments. Irradiation of the H-14 aldehydic signal increased the intensity of the H-1 proton by about 17%, and irradiation of the H-15 hydroxymethyl protons also produced a positive response at H-6, increasing the signal intensity by about 13%. These results also support the view that the 4,5-double bond has (*Z*)-configuration. These results show that ixerin A has the same medium ring system as 8-desoxyurospermal A. The stereochemistry of the lactone group was determined by the experiment described below. Reduction of 8-desoxyurospermal A with $NaBH_4$ in methanol gave the corresponding alcohol (V). Ixerin A was similarly reduced to give (V) [IR, ^{1}H-NMR comparisons]. The $NaBH_4$ reduction of α-methylene-γ-lactone usually affords 11,13-α-dihydro-lactone. Most naturally occurring sesquiterpenes with an 11,13-dihydro-lactone moiety have an α-oriented methyl group. On the basis of Narayanan's rule, we could deduce the stereochemistry to some extent.[3] This rule says that the signal of a pseudo-*eq* methyl group exhibits 0.23 ± 0.06 ppm upfield shift whereas that of a pseudo-*ax* one is 0.46 ± 0.06 ppm. The methyl group of ixerin A exhibited an upfield shift of 0.18 ppm in benzene-d_6 relative to chloroform-d_1 solution. Thus, the methyl group should be α-oriented.

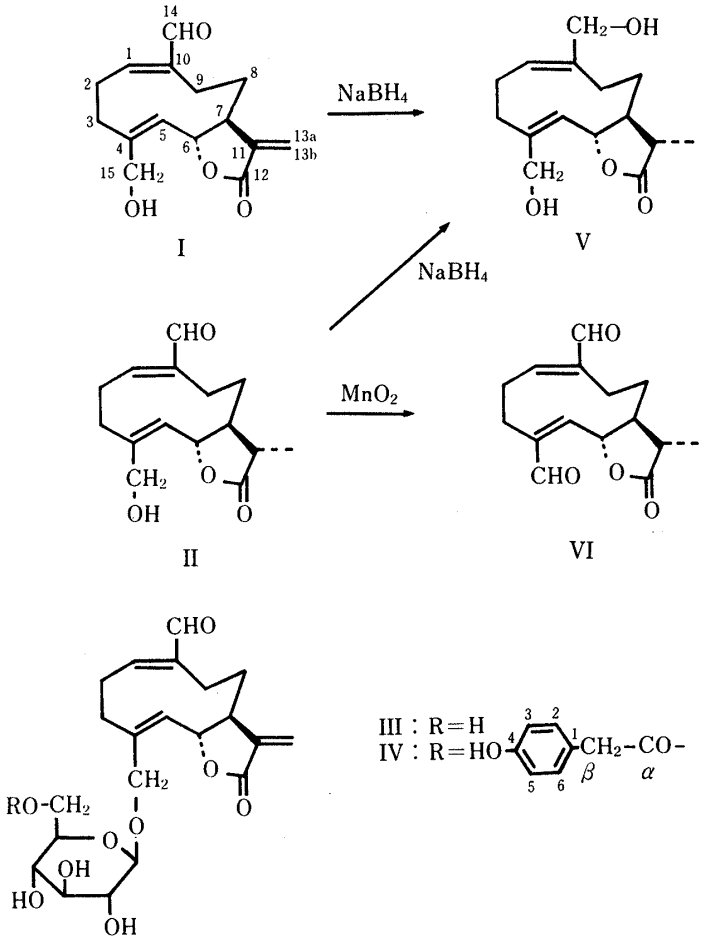

Chart 1

TABLE I. ^{1}H-NMR Data for I, II, V, VI in $CDCl_3$

	I	II[a)]	V	VI
1	6.54 (br t, J=8.5 Hz)	6.46 (br t, J=8.5 Hz)	5.50 (br t, J=8.5 Hz)	6.58 (br t, J=8 Hz)
2 3	1.5—3.0 (m)	1.2—3.0 (m)	1.4—2.7 (m)	1.5—2.5 (m)
5	5.15 (d, J=10 Hz)	5.00 (d, J=10 Hz)	5.10 (d, J=10 Hz)	6.15 (d, J=10 Hz)
6	4.81 (t, J=10 Hz)	5.36 (t, J=10 Hz)	4.85 (t, J=10 Hz)	5.48 (t, J=10 Hz)
7, 8, 9	1.5—3.0 (m)	1.2—3.0 (m)	1.4—2.7 (m)	1.5—2.5 (m)
11	—	1.2—3.0 (m)	1.4—2.7 (m)	1.5—2.5 (m)
13a 13b	5.48 (d, J=3.3 Hz) 6.14 (d, J=3.3 Hz)	1.13 (d, J=7 Hz)	1.22 (d, J=7 Hz)	1.20 (d, J=7 Hz)
14	9.42 (br s)	9.55 (br s)	4.00—4.60 (m)	9.50 (br s)
15	4.10, 4.45 (2H, AB-type, J=13 Hz)	4.15, 4.45 (2H, AB-type, J=13 Hz)	4.00—4.60 (m)	10.17 (br s)

a) Pyridine-d_5.

TABLE II. ^{13}C-NMR Data for I, II, III, IV, V in Pyridine-d_5

	I	II[a)]	III	IV	V[b)]
Aglycone moiety					
1	153.7	153.0	153.4	153.4	126.2
2	27.3	27.0	27.1	27.4	26.3
3	33.2	32.7	33.5	33.9	34.7
4	142.1	138.8	138.4	138.3	142.4
5	127.7	129.2	129.7	130.0	128.5
6	79.6	78.8	79.4	79.6	80.4
7	46.1	39.7	46.1	46.1	42.8
8	22.1[c)]	22.1[d)]	22.1[e)]	22.1[f)]	27.0
9	24.3[c)]	22.4[d)]	24.1[e)]	24.2[f)]	24.8
10	144.9	145.2	145.0	145.0	141.4
11	140.8	44.7	140.5	140.6	50.6
12	170.3	179.9	170.1	170.2	181.5
13	118.1	10.6	118.2	118.2	13.0
14	196.1	196.0	196.1	196.1	66.4
15	60.4	60.6	67.8	68.1	60.8
Glucose moiety					
1			105.0	104.6	
2			75.0	75.4	
3			78.5	78.3	
4			71.6	71.2	
5			78.5	74.7	
6			62.8	64.7	
p-Hydroxyphenylacetic acid moiety					
α				172.1	
β				40.5	
1				125.2	
2				116.2	
3				130.9	
4				157.8	
5				130.9	
6				116.2	

a) $CDCl_3$. *b*) CD_3OD. *c*—*f*) May be interchangeable in each column.

Compound III, named ixerin B, was obtained from the more polar fraction of the column chromatography. The molecular formula was $C_{21}H_{28}O_9$. The IR spectrum showed strong absorptions at 3390 cm^{-1} (hydroxyl), 1755 cm^{-1} (lactone) and 1665 cm^{-1} (aldehyde). The ^{1}H-NMR signals were similar to those of 8-desoxyurospermal A, but in the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum, this compound showed signals due to glucose in addition to those of 8-desoxyurospermal A. The enzymic hydrolysis of III gave 8-desoxyurospermal A as the aglycone, and hydrolysis with 10% sulfuric acid gave glucose. These results led us to the conclusion that III is the glucoside of 8-desoxyurospermal A. The anomeric structure was determined from the C_1–H_1 coupling constant ($J=155$ Hz). It is well known that $J_{C_1-H_1}$ is 169—171 Hz for the α-anomer whereas the corresponding value for the β-anomer is 158—162 Hz.[4] Thus we determined the structure to be III.

Compound IV, named ixerin C, has the molecular formula $C_{29}H_{34}O_{11}$. The ultraviolet (UV) spectrum of IV exhibited absorption bands at 224.5 and 277.5 nm. Its IR spectrum showed absorptions at 3400 cm^{-1} (hydroxyl), 1750 cm^{-1} (lactone) and 1738 cm^{-1} (aldehyde). The ^{1}H-NMR spectrum of IV was nearly the same as that of III; the major difference was A_2B_2 type signals at δ 7.08 and 7.30. Treatment of IV with 2% sodium hydroxide gave *p*-hydroxyphenyl acetic acid and III, so that ixerin C has the *p*-hydroxyphenyl acetate group at the glucose moiety of III. A comparison of the ^{13}C-NMR spectrum of IV with that of III showed an upfield shift of 3.8 ppm at C-5 and downfield shift of 1.9 ppm at C-6 of glucose, suggesting that the *p*-hydroxyphenyl acetic acid was esterified at C-6 in the glucose moiety.

Experimental

Melting points were determined on a Yanaco MP-500 micro melting point apparatus and are uncorrected. Optical rotations were determined on a JASCO DIP-140 digital polarimeter and circular dichroism (CD) spectra were determined with a JASCO J-400 X spectropolarimeter.

IR spectra were run on a JASCO A-2 grating infrared spectrophotometer, UV spectra on a Shimadzu UV-360 recording spectrophotometer and MS on a JEOL JMS-D/100 mass spectrometer. NMR spectra were recorded on a JEOL FX-90 Q spectrometer (^{1}H-NMR: 89.55 MHz, ^{13}C-NMR: 22.5 MHz); chemical shifts are given in (ppm) with tetramethylsilane as an internal standard.

Isolation——Air-dried whole plants of *Ixeris tamagawaensis* (4.6 kg) were extracted with methanol under reflux. The extract was concentrated *in vacuo* and the residue was suspended in water. This suspension was extracted with ether and *n*-butanol to give gums, 150 and 90 g, respectively.

The ether extract gave no characteristic compound. The *n*-butanol extract was chromatographed on a polyamide column with H_2O–MeOH (3 : 1) and acetone as eluents. Luteolin-7-glucoside was obtained from the acetone eluate. The H_2O–MeOH (3 : 1) eluate was chromatographed on a silica gel column repeatedly to give several sesquiterpene lactones.

Luteolin-7-glucoside——Yellow needles (1.2 g). mp 250—253 °C, IR ν_{max}^{KBr} cm^{-1}: 3325, 1660, 1600. UV λ_{max}^{MeOH} nm (log ε): 254 (4.27), 265 (4.23), 347 (4.28). UV $\lambda_{max}^{MeOH+MeONa}$: 264 (4.30), 398 (4.32). UV $\lambda_{max}^{MeOH+AlCl_3}$: 273 (4.34), 298 (3.96), 330 (3.74), 427 (4.41). UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 270 (4.23), 295 (4.10), 356 (4.17), 386 (4.19). UV $\lambda_{max}^{MeOH+NaOAc}$: 258 (4.34), 404 (4.29). UV $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$: 258 (4.41), 371 (4.35). ^{1}H-NMR (pyridine-d_5) δ: 5.75 (1H, br d, $J=6.5$ Hz, anomeric proton), 6.75 (1H, d, $J=2$ Hz, H-6), 6.87 (1H, s, H-3), 6.92 (1H, d, $J=2$ Hz, H-8), 7.23 (1H, d, $J=10$ Hz, H-5′), 7.50 (1H, dd, $J=2$, 10 Hz, H-6′), 7.82 (1H, d, $J=2$ Hz, H-2′). This was identified by comparison with an authentic sample [mp. TLC, ^{1}H-NMR, IR].

8-Desoxyurospermal A (I)——Amorphous powder (120 mg). $[\alpha]_D^{23}$: −40.6° ($c=0.32$, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{18}O_4 \cdot 1/4H_2O$: C, 67.52; H, 6.80. Found: C, 67.82; H, 7.05. IR ν_{max}^{KBr} cm^{-1}: 3460, 1758, 1680. UV λ_{max}^{MeOH} nm (log ε): 224.0 (4.01). MS *m/z*: 262 (M^+), 244 ($M^+ - H_2O$), 233 ($M^+ - CHO$). CD ($c=1.07\times10^{-5}$, MeOH) [θ] (nm): −38000 (235).

Ixerin A (II)——Colorless prisms (72 mg). mp, 134—138 °C (MeOH). $[\alpha]_D^{23}$: −54.6° ($c=0.18$, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.11; H, 7.56. IR ν_{max}^{KBr} cm^{-1}: 3415, 1768, 1665. UV λ_{max}^{MeOH} nm (log ε): 229.5 (4.10). MS *m/z*: 246 ($M^+ - H_2O$), 235 ($M^+ - CHO$), CD ($c=1.04\times10^{-5}$, MeOH) [θ] (nm): −50800 (233).

Ixerin B (III)——Amorphous powder (250 mg). $[\alpha]_D^{23}$: −11.9° ($c=2.0$, MeOH). *Anal.* Calcd for $C_{21}H_{28}O_9$: C, 59.42; H, 6.65. Found: C, 59.37; H, 6.72. IR ν_{max}^{KBr} cm^{-1}: 3390, 1755, 1665. UV λ_{max}^{MeOH} nm (log ε): 226 (4.15). ^{1}H-NMR (pyridine-d_5) δ: 5.09 (1H, d, $J=11.0$ Hz, H-5), 5.33 (1H, d, $J=3.1$ Hz, H-13), 6.13 (1H, d, $J=3.5$ Hz, H-13), 6.39 (1H, br t, $J=8.5$ Hz, H-1), 9.54 (1H, s, H-14).

Ixerin C (IV)——Amorphous powder (400 mg). $[\alpha]_D^{23}$: +3.8° (c=0.40, MeOH). *Anal.* Calcd for $C_{29}H_{34}O_{11}$: C, 62.36; H, 6.14. Found: C, 62.10; H, 6.15. IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1738, 1664. UV λ_{max}^{MeOH} nm (log ε): 224.5 (4.32), 277.5 (3.75). ^{1}H-NMR (pyridine-d_5) δ: 3.68 (2H, s, Ar-C$\underline{H}_2$), 5.32 (1H, d, J=3.3 Hz, H-13a), 6.12 (1H, d, J=3.3 Hz, H-13b), 6.35 (1H, br t, J=8.5 Hz, H-1), 7.08 (2H, d, J=9.0 Hz, H-2′, 6′), 7.30 (2H, d, J=9.0 Hz, H-3′, 5′).

Reduction of I——8-Desoxyurospermal A (I) (18 mg) was stirred in MeOH with $NaBH_4$ (5 mg) for 10 min at 0 °C. Acetic acid was added and the whole was diluted with water, followed by extraction with ethyl acetate (AcOEt). The solution was concentrated to dryness to give the corresponding alcohol (V), which was purified on a silica gel column. Yield, 5 mg (colorless gum). IR ν_{max}^{KBr} cm^{-1}: 3425, 1750.

Oxidation of II——Ixerin A (II) (26 mg) was stirred in benzene with active MnO_2 (200 mg) for 48 h at room temperature. The solution was filtered and concentrated to dryness. Crystallization from AcOEt gave the dialdehyde (VI) (3 mg) as colorless needles. IR ν_{max}^{KBr} cm^{-1}: 1770, 1740, 1680.

Reduction of II——Ixerin A (II) (10 mg) was stirred in MeOH with $NaBH_4$ (5 mg) for 10 min at 0 °C. Acetic acid was added and the mixture was diluted with water, followed by extraction with AcOEt 3 times. The solution was concentrated to dryness and purified by silica gel column chromatography to give the corresponding alcohol (V) (5 mg). This was identical with the alcohol derived from I [^{1}H-NMR, IR comparisons].

Enzymic Hydrolysis of III——Ixerin B (III) (67 mg) was dissolved in water (3 ml) and stirred with hesperidinase for 36 h at 35 °C. The solution was extracted with AcOEt 3 times and chromatographed on silica gel to give an aglycone (20 mg), which was identical with I.

Saponification of IV——A solution of ixerin C (IV) (*ca.* 2 mg) in aqueous 2% sodium hydroxide was stirred for 3 h at room temperature under a nitrogen atmosphere. The solution was acidified with dilute hydrochloric acid and extracted with AcOEt and *n*-butanol 3 times. The AcOEt layer was concentrated to give *p*-hydroxyphenylacetic acid, which was detected by high performance liquid chromatography (HPLC) in comparison with an authentic sample. The *n*-butanol layer was concentrated to give ixerin B, which was detected by thin layer chromatography (TLC). HPLC conditions: column, 4 mm × 25 cm, Lichrosorb RP-8; eluent, H_2O–CH_3CN (75 : 25); detector, UV 240 nm.

Enzymic Hydrolysis of IV——Ixerin C (IV) (46 mg) and hesperidinase (40 mg) were dissolved in water and the solution was stirred for 40 h at 35 °C. The solution was extracted with AcOEt 3 times and chromatographed on silica gel to give an aglycone (16 mg). The aglycone was identical with 8-desoxyurospermal A (I) [^{1}H-NMR, IR comparisons].

Acid Hydrolysis of Ixerin B and Ixerin C——A solution of a glycoside (*ca.* 2 mg) in 10% sulfuric acid (1 ml) was heated in 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 sodium borohydride (*ca.* 3 mg) for 1 h. 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 reagent was evaporated off *in vacuo*. Glucitol acetate was detected by gas chromatography. Conditions: column, 1.5% OV-17, 3 mm × 1 m; column temperature, 230 °C; carrier gas, N_2; t_R, 3.8 min.

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