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# Sesquiterpene Lactones from *Ixeris tamagawaensis* KITAM. III[1)]

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Two new germacranolides, ixerins H and I, and three new melampolides, ixerins J, K and L were isolated from the polar and less polar fractions of *Ixeris tamagawaensis* KITAM., respectively. The structures and stereochemistry were established partly by chemical transformations and mainly by the use of $^{1}H$- and $^{13}C$- nuclear magnetic resonance spectroscopy.

**Keywords**——*Ixeris tamagawaensis*; Compositae; sesquiterpene; ixerin H; ixerin I; ixerin J; ixerin K; ixerin L

As a continuation of our search for sesquiterpene glycosides with potential biological activity in Compositae plants, we have examined *Ixeris tamagawaensis* KITAM. A chemical investigation of this rare plant led to the isolation of thirteen cytotoxic and antitumor-active sesquiterpene lactones, of which eight have already been reported. We now report the structure elucidation of these compounds by means of spectrometry; two of the compounds are the first sesquiterpenes bearing a germacranolide type skeleton to be isolated from this plant.

We will discuss first the structure elucidation of ixerin H (I), $C_{21}H_{32}O_8$. Its infrared (IR) spectrum showed strong absorptions at 3400 $cm^{-1}$ (hydroxyl) and 1740 $cm^{-1}$ (lactone). The $^{1}H$-nuclear magnetic resonance ($^{1}H$-NMR) spectrum showed a doublet methyl signal at $\delta$ 1.21 ($J=7$ Hz) and a broad singlet methyl signal at $\delta$ 1.35. On the other hand, in the $^{13}C$-nuclear magnetic resonance ($^{13}C$-NMR) spectrum, signals due to a glucosyl group were observed. Hydrolysis with hesperidinase gave the corresponding aglycone (II). The $^{1}H$-NMR spectrum of II was more informative as it showed an olefinic proton signal at $\delta$ 4.88 (br t, $J=8$ Hz), which was assigned to H-1 and furthermore there was an AB-type quartet signal ($J=13$ Hz) at $\delta$ 3.95 and 4.35, which was deduced to be due to the C-15 hydroxymethyl group. This aglycone moiety was identical with $\beta$-11,13-dihydro-8-desoxysalonitenolide [comparison of $^{1}H$-NMR, IR and mp].[2)] Acid hydrolysis of ixerin H gave glucose. The anomeric structure of ixerin H was determined to be $\beta$ from the $J_{C_1-H_1}$ value (154 Hz).[3)]

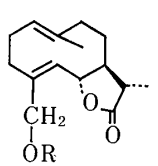
Ixerin I (III) has the molecular formula $C_{29}H_{36}O_{11}\cdot H_2O$ and its IR spectrum showed strong absorption at 3400 $cm^{-1}$ (hydroxyl), 1755 $cm^{-1}$ (lactone) and 1725 $cm^{-1}$ (ester). In the $^{1}H$-NMR spectrum, two characteristic doublets of exocyclic methylene were observed at $\delta$ 5.45 ($J=3.1$ Hz) and 6.32 ($J=3.7$ Hz). The signals of *para*-substituted $A_2B_2$-type aromatic protons appeared at $\delta$ 7.10 and 7.32 ($J=9$ Hz).

Because of the high polarity of ixerin I, we considered that it was a sesquiterpene glycoside, so that ixerin I (III) was hydrolyzed with crude hesperidinase in order to give the aglycone (IV). Detailed analysis of the $^{1}H$-NMR spectrum of IV and extensive spin-decoupling experiments enabled us to establish that ixerin I was a germacranolide-type sesquiterpene lactone. The stereochemistry of ixerin I was determined as follows. If the assumption is made that the absolute configuration of the C-7 side chain is $\beta$, as in all naturally occurring sesquiterpene lactones of authenticated stereochemistry, the large coupling constants ($J_{7-13}=3.1$ and 3.7 Hz) show that the lactone fusion is *trans* and that H-6 is $\beta$-oriented.[4)] The configurations of the two double bonds were determined mainly from the $^{13}C$

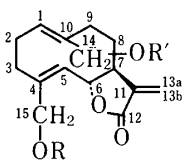
chemical shifts of C-2, C-3 and C-9. In the $^{13}$C-NMR spectrum, allylic methylene usually resonates at higher field in the *cis* isomer than in the *trans* isomer. In the case of a germacranolide-type sesquiterpene, which has a *trans* 1,10-double bond, the C-9 methylene carbon usually resonates below 30 ppm. In contrast, in the melampolide series compounds, having a *cis*-1,10-double bond, the C-9 carbon signal never appears below 30 ppm. On the other hand, the C-2 carbon atom was unaffected whether the 1,10-double bond was *trans* or not. This rule was adaptable to 4,5-double bond, that is to say, when the 4,5-double bond was *trans*, the C-3 carbon signal was observed at lower than 30 ppm. The C-9 and C-3 carbon signals were both observed below 30 ppm, so that ixerin I has a 1,10-*trans*-4,5-*trans* germacradiene type skeleton. The $^1$H-NMR chemical shifts supported this assumption.[5] The allylic alcohol *cis* and *trans* isomers can be distinguished in the $^1$H-NMR spectrum by the chemical shift of the $\beta$-vinyl proton because the $\beta$-vinyl proton is more sensitive to environmental changes than other protons and is thus preferred for stereochemical assignment. The H-1 signal in IV appeared at $\delta$ 5.08 (br t, $J$=8 Hz), but in compound V, which was derived from 8-desoxyurospermal A (XV),[6] the H-1 signal was observed at $\delta$ 5.65. Thus, the partial structure of ixerin I was established.

On the other hand, from the $^{13}$C-NMR and IR spectra, the existence of an ester carbonyl group was deduced. Saponification with 2% NaOH afforded *p*-hydroxyphenylacetic acid. Acid hydrolysis gave glucose and the anomeric structure was determined to be $\beta$ from the $J_{C_1-H_1}$ value ($J$=156 Hz). The alternative structure (VI) was excluded by the $^1$H-NMR chemical shift of the C-15 hydroxymethyl group. In the acetylated derivative (VII), the C-15 hydroxymethyl signal was shifted downfield, so that the glucose moiety was attached to C-15 and not to C-14.

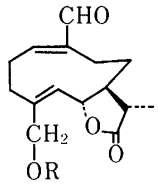
Ixerin J (VIII) was obtained from the more polar fraction of the *n*-butanol extract and it has the molecular formula $C_{21}H_{30}O_9 \cdot H_2O$, mp 113.5—115.5 °C. Its IR spectrum showed absorptions at 3370 cm$^{-1}$ (hydroxyl), 1750 cm$^{-1}$ (lactone) and 1672 cm$^{-1}$ (aldehyde). The $^1$H-NMR spectrum was nearly the same as that of ixerin B (IX), which was previously isolated in this laboratory,[6] but the most prominent difference was the absence of two doublets assigned to the exomethylene protons in the $\gamma$-lactone ring and the presence of a characteristic doublet methyl signal at $\delta$ 1.15 required the structure to be VIII. Subsequently, ixerin J was hydrolyzed with hesperidinase to afford an aglycone (X) and this was found to be identical with ixerin A which was previously isolated in this laboratory [comparison of $^1$H-NMR, $^{13}$C-NMR and IR].[6] Acid hydrolysis of ixerin J gave glucose. The anomeric structure of ixerin J was determined to be $\beta$ from the $J$ value of the anomeric proton ($J$=8 Hz).



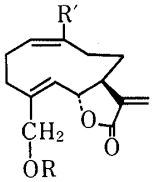
I: R=glucopyranosyl
II: R=H



| | R | R′ |
|---|---|---|
| III: | glucopyranosyl | *p*-hydroxyphenylacetyl |
| IV: | H | *p*-hydroxyphenylacetyl |
| VI: | *p*-hydroxyphenylacetyl | glucopyranosyl |
| VII: | acetyl | *p*-hydroxyphenylacetyl |



VIII: R=glucopyranosyl
X: R=H



| | R | R′ |
|---|---|---|
| V: | H | $CH_2$–OH |
| IX: | glucopyranosyl | CHO |
| XV: | H | CHO |

Chart 1

TABLE I. $^1$H-NMR Data for II, IV, V, VII, X in $CDCl_3$ (90 MHz)

| Proton | II | IV | V[a)] | VII | X[a)] |
|---|---|---|---|---|---|
| 1 | 4.88 (br t, $J$=8 Hz) | 5.08 (br t, $J$=8 Hz) | 5.65 (br t, $J$=8 Hz) | 5.12 (br t, $J$=8 Hz) | 6.41 (br t, $J$=8.5 Hz) |
| 2 | 1.4—2.7 (m) | 1.2—2.7 (m) | 1.2—2.8 (m) | 1.3—2.6 (m) | 1.2—3.0 (m) |
| 3 | 1.4—2.7 (m) | 1.2—2.7 (m) | 1.2—2.8 (m) | 1.3—2.6 (m) | 1.2—3.0 (m) |
| 5 | 4.75 (m) | 4.83 (d, $J$=10 Hz) | 5.1—5.2 (m) | 5.95 (d, $J$=10 Hz) | 5.02 (d, $J$=10 Hz) |
| 6 | 4.75 (m) | 4.47 (t, $J$=10 Hz) | 5.1—5.2 (m) | 4.41 (t, $J$=10 Hz) | 5.31 (t, $J$=10 Hz) |
| 7 | 1.4—2.7 (m) | 1.2—2.7 (m) | 1.2—2.8 (m) | 1.3—2.6 (m) | 1.2—3.0 (m) |
| 8 | 1.4—2.7 (m) | 1.2—2.7 (m) | 1.2—2.8 (m) | 1.3—2.6 (m) | 1.2—3.0 (m) |
| 9 | 1.4—2.7 (m) | 1.2—2.7 (m) | 1.2—2.8 (m) | 1.3—2.6 (m) | 1.2—3.0 (m) |
| 13a | 1.24 (d, $J$=7 Hz) | 5.46 (d, $J$=3.1 Hz) | 5.31 (d, $J$=3.0 Hz) | 5.48 (d, $J$=3.1 Hz) | 1.02 (d, $J$=7 Hz) |
| 13b | | 6.27 (d, $J$=3.7 Hz) | 6.16 (d, $J$=3.3 Hz) | 6.26 (d, $J$=3.7 Hz) | |
| 14 | 1.35 (br s) | 4.35 (AB-type, 4.50 q, $J$=13 Hz) | 4.2—4.7 (m) | 4.2—4.4 (m) | 9.58 (br s) |
| 15 | 3.95 (AB-type, 4.35 q, $J$=13 Hz) | 3.86 (AB-type, 4.10 q, $J$=13 Hz) | 4.2—4.7 (m) | 4.2—4.4 (m) | 4.43 (AB-type, 4.67 q, $J$=14 Hz) |
| Ac | | | | 2.12, 2.30 (s) | |
| *p*-Hydroxyphenylacetic acid moiety | | | | | |
| $\beta$ | | 3.57 (br s) | | | |
| 2,6 | | 6.73 (d, $J$=9 Hz) | | | |
| 3,5 | | 7.09 (d, $J$=9 Hz) | | | |

*a*) In pyridine-$d_5$.

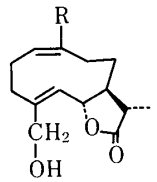
Ixerin K (XI) was obtained from the less polar fraction and has the molecular formula $C_{15}H_{22}O_4 \cdot 1/4H_2O$. Its IR spectrum showed absorptions at 3350 $cm^{-1}$ (hydroxyl) and 1750 $cm^{-1}$ (lactone) and the $^1$H-NMR spectrum exhibited a characteristic doublet methyl signal at $\delta$ 1.22 ($J$=7 Hz) as well as two olefinic protons at $\delta$ 5.10 (d, $J$=10 Hz) and $\delta$ 5.54 (br t, $J$=8 Hz). The former was coupled with the signal at $\delta$ 4.84 (t, $J$=10 Hz) which was assigned to H-6. The signals observed in the region of $\delta$ 4.0—4.6 (m) were assigned to C-14 and C-15 hydroxymethyl groups. From these spectral data, the structure of ixerin K was elucidated to be XI. Authentic ixerin A (X) was reduced with $NaBH_4$ to the corresponding alcohol (XI), which was identical with ixerin K [$^1$H-NMR, $^{13}$C-NMR, IR].

Ixerin L (XII) has the molecular formula $C_{15}H_{22}O_4 \cdot 1/4H_2O$ and the spectral data were similar to those of ixerin K, so that ixerin L is closely related with ixerin K. In the $^1$H-NMR spectrum, a doublet methyl signal was observed at $\delta$ 1.07 ($J$=7 Hz) and olefinic proton signals at $\delta$ 5.15 (d, $J$=9.8 Hz) and 5.69 (br t, $J$=8.5 Hz). A triplet signal which was assigned to H-6 was observed at $\delta$ 5.41 ($J$=10.1 Hz). At $\delta$ 4.34 and 4.41, AB-type signals were seen ($J$=13.4 Hz), in addition to AB-type signals at $\delta$ 4.49 and 4.73 ($J$=13.4 Hz), and these signals were assigned to C-14 and C-15 hydroxymethyl groups. The presence of two primary hydroxyl groups was supported by the transformation to the acetate (XIII). These data indicated that ixerin L had the germacradiene type skeleton and next we determined the configuration of the double bonds. Oxidation with activated $MnO_2$ afforded a dialdehyde (XIV), whose $^1$H-NMR spectrum enable us to conclude that the 1,10-double bond has (*E*)-configuration and the 4,5-double bond has (*Z*)-configuration because of the chemical shifts of the newly produced aldehydic protons, $\delta$ 9.48 and 10.17, which were assigned to H-14 and H-15 respectively, on the basis of nuclear Overhauser effect (NOE) experiments. Irradiation of the H-14 aldehydic proton signal increased the intensity of the H-1 signal about 9%, and irradiation of the H-15 aldehydic proton signal produced a positive response at the H-6 proton (about 18%). On the other hand, the solvent shift of the H-13 methyl signal for a pseudo-axial methyl group should be considerably greater than that for a pseudo-equatorial methyl group, which enables us to

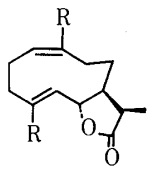
TABLE II. $^{13}$C-NMR Data for I, III, V, VIII in Pyridine-$d_5$

| Carbon | I | III | V | VIII |
|---|---|---|---|---|
| Aglycone moiety | | | | |
| 1 | 126.8 | 133.2 | 123.6 | 153.1 |
| 2 | 28.1[a)] | 28.1[a)] | 25.3[a)] | 25.2[a)] |
| 3 | 35.9 | 35.7 | 34.5 | 33.5 |
| 4 | 139.9 | 141.0 | 142.3 | 136.9 |
| 5 | 130.3 | 130.5 | 126.8 | 130.3 |
| 6 | 79.6 | 80.2 | 79.4 | 79.2 |
| 7 | 54.8 | 50.8 | 45.9 | 41.4 |
| 8 | 26.9[a)] | 27.1[a)] | 26.0[a)] | 27.0[a)] |
| 9 | 41.1 | 36.9 | 24.1[a)] | 22.4[a)] |
| 10 | 137.3 | 135.6 | 141.4 | 145.3 |
| 11 | 42.0 | 140.7 | 142.5 | 49.8 |
| 12 | 178.1 | 170.1 | 170.3 | 178.5 |
| 13 | 13.3 | 119.2 | 117.6 | 12.7 |
| 14 | 16.1 | 62.1 | 65.6 | 195.9 |
| 15 | 67.6 | 67.7 | 60.6 | 67.7 |
| Glucose moiety | | | | |
| 1 | 105.0 | 104.6 | | 105.1 |
| 2 | 75.0 | 74.9 | | 75.0 |
| 3 | 78.3 | 78.3 | | 78.5 |
| 4 | 71.6 | 71.6 | | 71.6 |
| 5 | 78.2 | 78.3 | | 78.5 |
| 6 | 62.8 | 62.8 | | 62.7 |
| *p*-Hydroxyphenylacetic acid moiety | | | | |
| α | | 172.1 | | |
| β | | 41.0 | | |
| 1 | | 125.0 | | |
| 2 | | 116.3 | | |
| 3 | | 130.9 | | |
| 4 | | 157.9 | | |
| 5 | | 130.9 | | |
| 6 | | 116.3 | | |

*a*) May be interchangeable in each column.



X: R=CHO
XI: R=$CH_2$–OH



XII: $CH_2$–OH
XIII: $CH_2$–O–C(=O)–$CH_3$
XIV: CHO

Chart 2

determine the orientation of the C-11 methyl group. In the present instance, the upfield shifts of H-13 for XI and XII were 0.25 and 0.40 ppm, respectively, so that the methyl group was β-oriented if Narayanan's empirical rule holds in the case of ixerin L.[7] The stereochemistry of the γ-lactone ring was determined by extensive NOE experiments. Irradiation at the H-13 methyl signal produced an 11% enhancement in the intensity of the H-6 signal and irradiation at the H-7 signal, which overlapped with two unassignable proton signals, resulted in 20% enhancement of the signal intensity of H-5. These results clearly indicate that the C-4 methyl group, H-6 proton and C-11 methyl group have the same direction in space, and that the H-5

TABLE III. $^1$H-NMR Data for XI, XII, XIII, XIV in $CDCl_3$ (90 MHz)

| Proton | XI | XII[a] | XIII | XIV |
|---|---|---|---|---|
| 1 | 5.54 (br t, $J$=8 Hz) | 5.69 (br t, $J$=8.5 Hz) | 5.54 (br t, $J$=8 Hz) | 6.55 (br t, $J$=8 Hz) |
| 2 | 1.4—2.7 (m) | 1.5—2.7 (m) | 1.5—2.7 (m) | 1.3—2.8 (m) |
| 3 | 1.4—2.7 (m) | 1.5—2.7 (m) | 1.5—2.7 (m) | 1.3—2.8 (m) |
| 5 | 5.10 (d, $J$=10 Hz) | 5.15 (d, $J$=9.8 Hz) | 5.22 (d, $J$=10 Hz) | 6.13 (d, $J$=10 Hz) |
| 6 | 4.84 (t, $J$=10 Hz) | 5.41 (t, $J$=10.1 Hz) | 4.88 (t, $J$=10 Hz) | 5.45 (t, $J$=10 Hz) |
| 7 | 1.4—2.7 (m) | 2.25 (m) | 1.5—2.7 (m) | 1.3—2.8 (m) |
| 8 | 1.4—2.7 (m) | 1.5—2.7 (m) | 1.5—2.7 (m) | 1.3—2.8 (m) |
| 9 | 1.4—2.7 (m) | 1.5—2.7 (m) | 1.5—2.7 (m) | 1.3—2.8 (m) |
| 13 | 1.22 (d, $J$=7 Hz) | 1.07 (d, $J$=7.0 Hz) | 1.20 (d, $J$=7 Hz) | 1.08 (d, $J$=7 Hz) |
| 14 | 4.0—4.6 (m) | 4.49 (AB-type, 4.73 q, $J$=13.4 Hz)[b] | 4.76 (br s) | 9.48 (d, $J$<1 Hz) |
| 15 | 4.0—4.6 (m) | 4.34 (AB-type, 4.41 q, $J$=13.4 Hz)[b] | 4.40 (AB-type, 4.65 q, $J$=13 Hz) | 10.17 (s) |
| Ac | | | 2.08, 2.12 (s) | |

*a*) In pyridine-$d_5$ at 400 MHz.
*b*) May be interchangeable.

TABLE IV. $^{13}$C-NMR Data for XI, XII in Methanol-$d_4$

| Carbon | XI | XII |
|---|---|---|
| 1 | 126.2 | 126.0 |
| 2 | 26.3 | 26.3 |
| 3 | 34.7 | 34.7 |
| 4 | 142.4 | 142.6 |
| 5 | 128.5 | 128.9 |
| 6 | 80.4 | 80.7 |
| 7 | 42.8 | 41.1 |
| 8 | 27.0 | 23.5 |
| 9 | 24.8 | 24.9 |
| 10 | 141.4 | 141.0 |
| 11 | 50.6 | 45.4 |
| 12 | 181.5 | 182.6 |
| 13 | 13.0 | 10.9 |
| 14 | 66.4 | 66.2 |
| 15 | 60.8 | 60.7 |

olefinic proton and H-7 proton are on the same side of the plane, which can be rationalized only if the stereochemistry is as shown in XII.

## 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-40 spectropolarimeter. IR spectra were run on a JASCO A-202 grating infrared spectrophotometer, and mass spectra (MS) were measured on a JEOL JMS-D 100 mass spectrometer. Field desorption (FD) MS were measured on a JEOL JMS-D 300 instrument with a MS-FD 03 ion source. NMR spectra were recorded on JEOL FX 90 Q and JEOL GX 400 spectrometers ($^1$H-NMR 89.55 MHz and 399.65 MHz, $^{13}$C-NMR 22.5 MHz); chemical shifts are given in ppm based on tetramethylsilane as an internal standard.

**Isolation**——Whole plants of *Ixeris tamagawaensis* (7.2 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 ethyl acetate and with *n*-butanol to give gums, 106 and 46 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 sesquiterpenes.

**Ixerin H (I)**——Amorphous powder (50 mg). $[\alpha]_D^{25}$ +43.6° ($c$=0.51, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400, 1740. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.21 (3H, d, $J$=7 Hz, H-13), 1.35 (3H, br s, H-14). FD-MS $m/z$: 414 ($M^+$+2, 100), 413 ($M^+$+1, 25), 250 ($M^+$−162, 45).

**Ixerin I (III)**——Amorphous powder (100 mg). $[\alpha]_D^{25}$ +8.9° ($c$=0.36, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400, 1755, 1725. *Anal.* Calcd for $C_{29}H_{36}O_{11}\cdot H_2O$: C, 60.36; H, 6.55. Found: C, 60.26; H, 6.31. $^1$H-NMR (pyridine-$d_5$) $\delta$: 3.72 (2H, s, Ar–$CH_2$), 5.45 (1H, d, $J$=3.1 Hz, H-13a), 6.32 (1H, d, $J$=3.7 Hz, H-13b), 7.10 (2H, d, $J$=9 Hz, H-2, H-6 of ester), 7.32 (2H, d, $J$=9 Hz, H-3, H-5 of ester). CD ($c=1.8\times10^{-3}$, MeOH) [$\theta$] (nm): +5400 (259); ($c=1.8\times10^{-4}$, MeOH) [$\theta$] (nm): −123000 (222). FD-MS $m/z$: 561 ($M^+$+1).

**Ixerin J (VIII)**——Colorless prisms (15 mg). mp 113.5—115.5 °C (MeOH–AcOEt). $[\alpha]_D^{25}$ −54.0° ($c$=0.68, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3370, 1750, 1672. *Anal.* Calcd for $C_{21}H_{30}O_9\cdot H_2O$: C, 56.75; H, 7.26. Found: C, 56.90; H, 7.30. $^1$H-NMR (pyridine-$d_5$) $\delta$: 1.15 (3H, d, $J$=7 Hz, $CH_3$), 4.97 (1H, d, $J$=8 Hz, anomeric H), 6.36 (1H, br t, $J$=8.5 Hz, H-1), 9.54 (1H, br s, H-14). FD-MS $m/z$: 427 ($M^+$+1).

**Ixerin K (XI)**——Amorphous powder (8 mg). $[\alpha]_D^{25}$ −40.6° ($c$=0.62, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3350, 1750. *Anal.* Calcd for $C_{15}H_{22}O_4\cdot 1/4H_2O$: C, 66.52; H, 8.37. Found: C, 66.47; H, 8.20. MS $m/z$: 266 ($M^+$), 262 ($M^+$−4), 248 ($M^+-H_2O$), 235 ($M^+-CH_2OH$), 230 ($M^+-2H_2O$). CD ($c=1.3\times10^{-4}$, MeOH) [$\theta$] (nm): −124000 (206). $^1$H-NMR: Table III.

**Ixerin L (XII)**——Amorphous powder (5 mg). $[\alpha]_D^{25}$ +23.5° ($c$=0.37, MeOH). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3370, 1750. *Anal.* Calcd for $C_{15}H_{22}O_4\cdot 1/4H_2O$: C, 66.52; H, 8.37. Found: C, 66.25; H, 8.24. MS $m/z$: 264 ($M^+$−2), 248 ($M^+-H_2O$), 235 ($M^+-CH_2OH$), 230 ($M^+-2H_2O$). $^1$H-NMR: Table III.

**Enzymic Hydrolysis of I**——Ixerin H (25 mg) was dissolved in water (3 ml) and treated with crude hesperidinase for 5 h at 35 °C with stirring. This solution was extracted with ethyl acetate 3 times and the product was purified by silica gel column chromatography to afford an aglycone (12 mg), which was recrystallized from hexane–ether (colorless needles, mp 146—147 °C, 6 mg). CD ($c=7.2\times10^{-4}$, MeOH) [$\theta$] (nm): +22000 (233). $^1$H-NMR: Table I. MS $m/z$: 250 ($M^+$), 232 ($M^+-H_2O$), 219 ($M^+-CH_2OH$).

**Enzymic Hydrolysis of III**——Ixerin I (III) (15 mg) was dissolved in water and treated with crude hesperidinase (10 mg) for 2 h at 35 °C with stirring. The solution was extracted with ethyl acetate 3 times and the product was purified by silica gel column chromatography to give an aglycone (IV) (amorphous powder, 8 mg). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400, 1755, 1725. MS $m/z$: 398 ($M^+$), 380 ($M^+-H_2O$), 362 ($M^+-2H_2O$). $^1$H-NMR: Table I.

**Acetylation of IV**——IV (4 mg) was dissolved in acetic anhydride and pyridine (0.5 ml, each) and the mixture was left for 12 h, then concentrated *in vacuo* to give the acetate VII (5 mg). This product was purified by silica gel column chromatography (amorphous powder, 3 mg). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1763, 1740, 1725, 1660. MS $m/z$: 482 ($M^+$), 440 ($M^+-CH_2=C=O$), 423 ($M^+-2CH_2=C=O$), 289 [$M^+$−(ester side chain)].

**Saponification of III**——A solution of ixerin I (2 mg) in aqueous 2% NaOH was stirred for 3 h at room temperature under a nitrogen atmosphere. The solution was acidified with diluted HCl and extracted with ethyl acetate 3 times. The extract was concentrated to give *p*-hydroxyphenylacetic acid, which was shown by high performance liquid chromatography (HPLC) to be identical with a standard sample. HPLC conditions: column, Lichrosorb RP-8, 4 mm × 25 cm; eluent, $H_2O$–$CH_3CN$ (75 : 25); detector, UV 240 nm.

**Reduction of XV**——8-Desoxyurospermal A (XV) (10 mg) was dissolved in dry tetrahydrofuran (2 ml) and the solution was stirred with LiAl(*tert*-BuO)$_3$H (3 mg) for 17 h at room temperature. The residual reagent was destroyed with ethanol and the reaction mixture was diluted with excess water then extracted with AcOEt. The product was purified on a silica gel column to give V (3 mg). CD ($c=6.32\times10^{-4}$, MeOH) [$\theta$] (nm): −3090 (257).

**Enzymic Hydrolysis of VIII**——Ixerin J (VIII) (15 mg) was dissolved in water and treated with hesperidinase (3 mg) for 3 h at 35 °C with stirring. The solution was extracted with ethyl acetate 3 times and purified by silica gel column chromatography to afford the aglycone (X) (7 mg), which was recrystallized from methanol (colorless prisms, mp 135—136 °C). This product was identical with ixerin A [$^1$H-NMR, IR, mp]. $^1$H-NMR: Table I.

**Reduction of X**——Ixerin A (X) (7 mg) was dissolved in methanol (3 ml) and the solution was stirred with $NaBH_4$ (5 mg) for 10 min at room temperature. A small amount of acetic acid was added to destroy the reagent and excess water was added. This reaction product was passed through an Amberlite XAD-2 column, which was washed with water, then eluted with methanol. The methanol was evaporated off *in vacuo* and the residue was purified by silica gel column chromatography to afford the product (XI) (5 mg). This was identical with ixerin K [$^1$H-NMR, IR].

**Acetylation of XII**——Ixerin L (XII) (2 mg) was dissolved in pyridine and acetic anhydride (0.5 ml, each) and the mixture was left for 12 h at room temperature, then concentrated *in vacuo* to afford the acetate XIII (amorphous powder, 2 mg). $^1$H-NMR: Table III.

**Oxidation of XIII**——Ixerin L (XIII) (3 mg) was dissolved in chloroform (1 ml) and activated $MnO_2$ (50 mg) was added. The mixture was stirred for 24 h at room temperature. The reagent was filtered off and the filtrate was concentrated to give the dialdehyde (XIV) (amorphous powder, 3 mg). $^1$H-NMR: Table III.

**Acid Hydrolysis of Glycosides**——A solution of a glycoside (*ca.* 1 mg) in 10% $H_2SO_4$ (1 ml) was heated in a boiling water bath for 1 h. 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 30 min. 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*. From each glycoside, 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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## References and Notes

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