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Sesquiterpene Lactones from *Ixeris stolonifera* A. GRAY

KEIICHI NISHIMURA, TOSHIO MIYASE,* AKIRA UENO,
TADATAKA NORO, MASANORI KUROYANAGI,
and SEIGO FUKUSHIMA

Shizuoka College of Pharmacy, 2-2-1, Oshika, Shizuoka 422, Japan

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Eight new guaianolide type glycosides, ixerins M, N, O, P, Q, R, S and T, in addition to 8-epidesacylcynaropicrin-glucoside and macrocliniside A, have been isolated from the methanol extract of *Ixeris stolonifera* A. GRAY (Compositae). The structures of the new compounds were determined on the basis of chemical and spectral data.

Keywords——*Ixeris stolonifera*; Compositae; ixerin M; ixerin N; ixerin O; ixerin P; ixerin Q; ixerin R; ixerin S; ixerin T

In the course of a search for sesquiterpene lactone glycosides in *Ixeris* plants,[1] we have investigated the constituents of *Ixeris stolonifera* A. GRAY, and isolated eight new guaianolide-type glycosides, ixerins M, N, O, P, Q, R, S and T, which taste strongly bitter, along with 8-epidesacylcynaropicrin-glucoside[2] and macrocliniside A.[3]

8-Epidesacylcynaropicrin-glucoside (I), $C_{21}H_{28}O_9 \cdot 1/2\,H_2O$, $[\alpha]_D^{21}$ $-31.7°$, was shown to have an α-methylene-γ-lactone group by its infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra.[4] Enzymatic hydrolysis of I with crude hesperidinase afforded an aglycone (Ia). Compound Ia was shown to be identical with integrifolin, which had been isolated from *Andryala integrifolia* L.,[5] by comparing the ^{1}H-NMR spectra. Acetylation of I afforded a pentaacetate (Ib), whose ^{1}H-NMR and IR spectra and melting point (mp) were identical with those of 8-epidesacylcynaropicrin-glucoside acetate.[2] These results led us to identify I as 8-epidesacylcynaropicrin-glucoside.

Ixerin M (II), $C_{26}H_{36}O_{11} \cdot 1/2\,H_2O$, $[\alpha]_D^{19}$ $-40.6°$, showed absorptions at 1740 cm^{-1} (ester) and 1760 cm^{-1} (γ-lactone) in the IR spectrum. The ^{1}H-NMR spectrum was similar to that of I except for a methyl signal at δ 1.07 (d, $J=7$ Hz), integrated as 6H. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum was also similar to that of I, but five additional signals were observed. The signals of C-7 and C-9 were shifted upfield to δ 48.1 ($\Delta -1.9$ ppm) and 40.3 ($\Delta -3.4$ ppm), respectively, and that of C-8 was shifted downfield to δ 68.4 ($\Delta +2.4$ ppm). Thus, II was assumed to have a C_5 unit ester group at C-8. The ester moiety was shown to consist of a carboxylic ester (δ 174.1, s), two methines (δ 76.1, d; 32.5, d), one of which is linked with a hydroxyl group, and two methyl groups (δ 19.2, q; 17.2, q), by comparing the ^{13}C-NMR spectrum of II with that of I.

Acetylation of II afforded a pentaacetate (IIa), whose ^{13}C-NMR spectrum showed that the signals of C-1 and C-3 in the ester moiety were shifted upfield to δ 170.0 ($\Delta -4.1$ ppm) and 30.1 ($\Delta -2.4$ ppm) while that of C-2 was shifted downfield to δ 77.2 ($\Delta +1.1$ ppm). On the basis of these data, II was presumed to be the α-hydroxyisovalerate of I.

Saponification of II with alkali gave I and α-hydroxyisovaleric acid (A), whose *p*-bromophenacyl ester was identified by direct comparison [thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC)] with a synthetic sample. Acid hydrolysis of II gave glucose. These results led to the structure II for ixerin M.

Ixerin N (III), $C_{27}H_{38}O_{11} \cdot H_2O$, $[\alpha]_D^{19}$ $-43.5°$, showed an IR spectrum very similar to that of II. The ^{1}H-NMR spectrum was also similar to that of II except for the signals at δ 0.85 (3H, t, $J=7$ Hz) and at δ 1.2—1.7 (1H, m). In the ^{13}C-NMR spectrum, the signals were superimposable on those of II except for the ester moiety (Table I). The ester moiety consists of an ester carbonyl (δ 174.1, s), two methines (δ 75.5, d; 39.1, d), one of which has a hydroxyl group, a methylene (δ 24.5, t) and two methyl groups (δ 15.7, q; 11.6, q). The ^{13}C-NMR spectrum of IIIa showed similar acylation shifts to those of IIa in the ester moiety, so ixerin N (III) was assumed to be the α-hydroxy-β-methyl-*n*-valerate of I. This was confirmed by the same method as used for II.

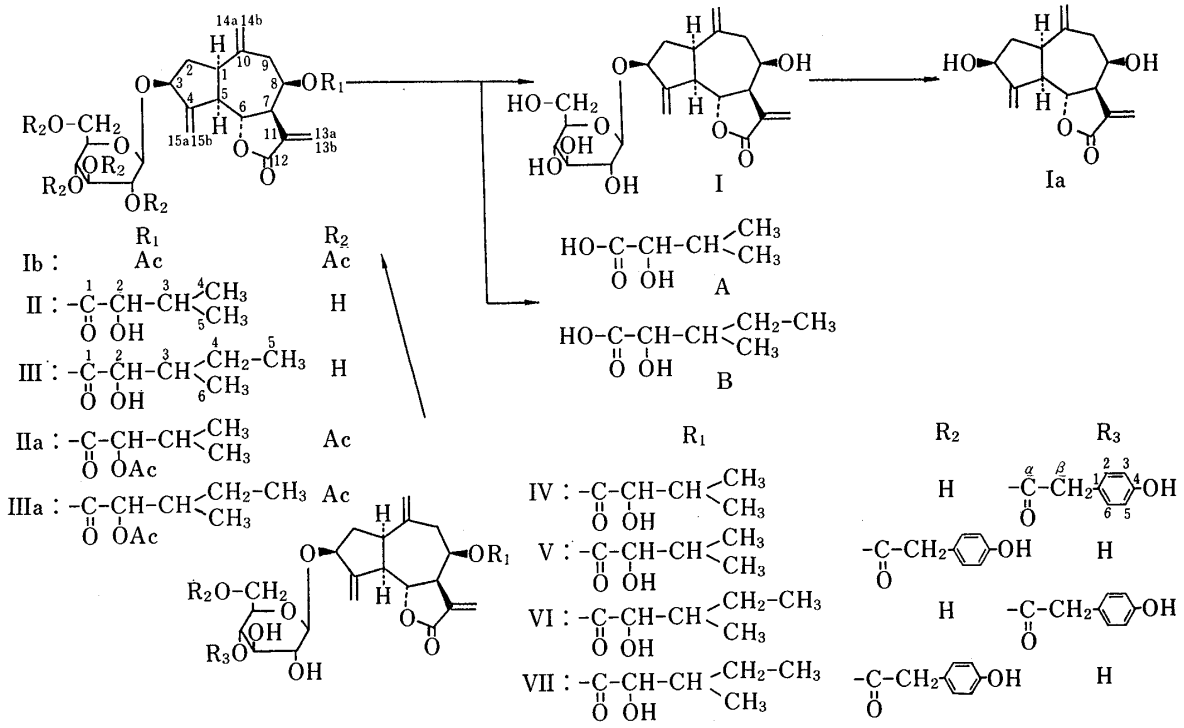


Chart 1

Ixerins O (IV), P (V), Q (VI), and R (VII) had an absorption maximum at 277.5 nm in the ultraviolet (UV) spectra. The ^{1}H-NMR spectra of IV and V were nearly the same as that of II, and those of VI and VII shown close similarity to that of III. These compounds, however, showed A_2B_2 type signals in the aromatic region (Table IV). On treatment with 1% barium hydroxide, IV and V gave *p*-hydroxyphenylacetic acid and II, while VI and VII gave *p*-hydroxyphenylacetic acid and III. In the ^{13}C-NMR spectrum of IV, the signals due to C-3 and C-5 in the glucose moiety were shifted upfield and that of C-4 was shifted downfield as compared with those of II (Table III). These results suggest that *p*-hydroxyphenylacetic acid was esterified at C-4 in the glucose moiety.[6] In V, VI and VII the positions of the aliphatic ester group were determined on the basis of acylation shifts, as in the case of II. Based on these data, we assigned structures IV, V, VI and VII to ixerins O, P, Q and R, respectively.

Ixerin S (VIII), $[\alpha]_D^{19}$ $+11.6°$, has the molecular formula $C_{26}H_{36}O_{11} \cdot 1/2\,H_2O$. The IR spectrum suggested the presence of hydroxyl groups (3420 cm^{-1}), an unsaturated γ-lactone (1760 cm^{-1}) and double bonds (1660, 1640 cm^{-1}). The ^{1}H-NMR spectrum of VIII was similar to that of II except for the absence of the exocyclic methylene proton signals of C-15, and the appearance of vinylogous methyl signals at δ 1.86. The triplet signal at δ 4.58 (1H, $J=9$ Hz) due to the lactonic methine proton at C-6 indicates *trans*-diaxial dispositions among H-5(α), H-6(β) and H-7(α), a feature common to all the lactones of this genus. The signal of this

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

Carbon No.	I	II	IIa	III	IIIa
Aglycone moiety					
1	45.1	44.9	45.2	44.8	45.1
2	38.5	38.4	37.9	38.4	37.9
3	80.7	80.7	81.2	80.7	81.3
4	150.8	150.3	150.3	150.4	150.5
5	50.7	50.4	51.3	50.3	51.2
6	78.5	78.9	78.5	78.8	78.6
7	50.0	48.1	48.3	48.1	48.3
8	66.0	68.4	69.4	68.4	69.5
9	43.7	40.3	40.3	40.3	40.6
10	144.9	143.7	143.7	143.7	143.1
11	137.6	136.2	135.6	136.2	135.6
12	170.1	169.1	170.5[c]	169.1	169.2
13	121.0	121.3	122.3	121.3	122.3
14	116.1	117.2	117.9	117.2	118.0
15	111.9	112.3	112.8	112.2	112.6
Sugar moiety					
1	104.5 (156 Hz)	104.1 (159 Hz)	100.6	104.1 (156 Hz)	100.8
2	75.1	75.2	72.2[b]	75.1	72.3[e]
3	78.3[a]	78.2	72.6[b]	78.5[d]	73.6[e]
4	71.8	71.9	68.9	71.9	69.0
5	78.0[a]	78.2	72.2[b]	78.1[d]	72.3[e]
6	62.9	63.0	62.5	63.0	62.6
Ester moiety					
1		174.1	170.0[c]	174.1	170.2
2		76.1	77.2	75.5	76.8
3		32.5	30.1	39.1	36.6
4		17.2	17.3	24.5	24.9
5		19.2	18.7	11.6	11.5
6				15.7	15.4

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

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

Proton No.	I	II	III
Aglycone moiety			
13a	5.70 (1H, d, J=3.1 Hz)	5.57 (1H, d, J=3.1 Hz)	5.59 (1H, d, J=3.1 Hz)
13b	6.50 (1H, d, J=3.3 Hz)	6.36 (1H, d, J=3.3 Hz)	6.30 (1H, d, J=3.3 Hz)
14a	5.16 (1H, br s)	5.19 (1H, br s)	5.20 (1H, br s)
14b	4.98 (1H, br s)	4.94 (1H, br s)	4.96 (1H, br s)
15a	5.88 (1H, br s)	5.84 (1H, br s)	5.86 (1H, br s)
15b	5.60 (1H, br s)	5.52 (1H, br s)	5.53 (1H, br s)
Ester moiety			
4		1.07 (6H, d, J=7 Hz)	
5		1.07 (6H, d, J=7 Hz)	0.85 (3H, t, J=7 Hz)
6			1.05 (3H, d, J=7 Hz)

Run at 89.55 MHz in pyridine-d_5 solution.

proton (H-6) was shifted downfield to δ 4.58 (Δ+0.48 ppm) in comparison with that of zaluzanin C.[7] Thus, H-6 and the C-8 hydroxyl group are in a 1,3-diaxial relation.

Enzymatic hydrolysis of VIII with crude hesperidinase afforded a ketonic aglycone

TABLE III. ^{13}C-NMR Chemical Shifts of IV, V, VI, and VII

Carbon No.	IV	V	VI	VII	Carbon No.	IV	V	VI	VII
Aglycone moiety					Ester moiety				
1	44.9	45.0	44.8	44.8	1	174.1	174.2	174.0	174.1
2	38.3	38.6	38.2	38.4	2	76.1[a]	75.3[b]	75.5[c]	75.5[d]
3	80.8	81.2	80.7	81.1	3	32.5	32.5	39.1	39.1
4	150.3	150.2	150.2	150.5	4	17.2	17.3	24.5	24.6
5	50.5	50.5	50.4	50.4	5	19.2	19.3	11.6	11.6
6	78.9	79.0	78.8	79.0	6			15.7	15.7
7	48.2	48.2	48.1	48.1	*p*-Hydroxyphenylacetic acid moiety				
8	68.4	68.5	68.5	68.5	α	171.8	172.0	171.7	172.0
9	40.3	40.7	40.2	40.7	β	40.9	40.7	40.8	40.7
10	143.7	144.0	143.6	143.8	1	125.3	125.4	125.2	125.3
11	136.2	136.3	136.2	136.2	2	131.0	131.0	130.9	130.8
12	169.3	169.3	169.1	169.3	3	116.2	116.3	116.2	116.2
13	121.4	121.4	121.3	121.4	4	157.4	158.0	157.8	157.8
14	117.3	117.3	117.2	117.2	5	116.2	116.3	116.2	116.2
15	112.5	112.3	112.4	112.2	6	131.0	131.0	130.9	130.8
Sugar moiety									
1	104.1	104.6	104.1	104.4					
2	75.5[a]	75.2[b]	75.4[c]	75.0[d]					
3	75.7[a]	78.3	75.9[c]	78.2					
4	73.0	71.8	72.9	71.7					
5	76.0[a]	76.1	75.6[c]	76.0					
6	62.3	65.1	62.1	65.0					

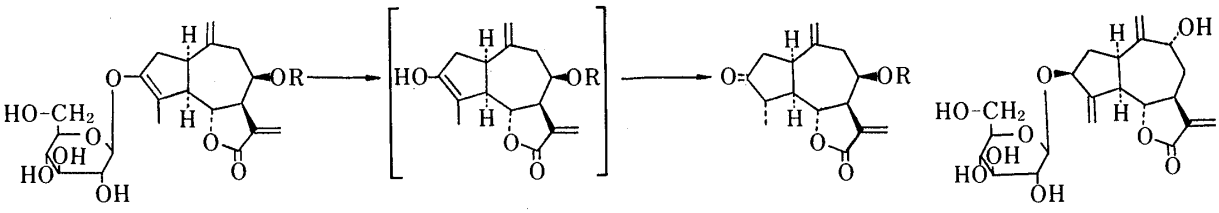
Run at 22.5 MHz in pyridine-d_5 solution. *a—d*) Assignments may be interchanged in each column.

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

Proton No.	IV	V	VI	VII
Aglycone moiety				
13a	5.57 (1H, d, J=3.1 Hz)	5.57 (1H, d, J=3.1 Hz)	5.57 (1H, d, J=3.1 Hz)	5.56 (1H, d, J=3.1 Hz)
13b	6.30 (1H, d, J=3.3 Hz)	6.28 (1H, d, J=3.3 Hz)	6.30 (1H, d, J=3.3 Hz)	6.27 (1H, d, J=3.3 Hz)
14a	5.18 (1H, br s)	5.23 (1H, br s)	5.18 (1H, br s)	5.24 (1H, br s)
14b	4.96 (1H, br s)	4.97 (1H, br s)	4.94 (1H, br s)	4.96 (1H, br s)
15a	5.84 (1H, br s)	5.81 (1H, br s)	5.82 (1H, br s)	5.79 (1H, br s)
15b	5.54 (1H, br s)	5.56 (1H, br s)	5.33 (1H, br s)	5.51 (1H, br s)
Ester moiety				
4	1.06 (6H, d, J=7 Hz)	1.06 (6H, d, J=7 Hz)		
5	1.06 (6H, d, J=7 Hz)	1.06 (6H, d, J=7 Hz)	0.86 (3H, t, J=7 Hz)	0.86 (3H, t, J=7 Hz)
6			1.06 (3H, d, J=7 Hz)	1.04 (3H, d, J=7 Hz)
p-Hydroxyphenylacetic acid moiety				
2, 6	7.34 (2H, d, J=9 Hz)	7.35 (2H, d, J=9 Hz)	7.34 (2H, d, J=9 Hz)	7.34 (2H, d, J=9 Hz)
3, 5	7.08 (2H, d, J=9 Hz)	7.11 (2H, d, J=9 Hz)	7.06 (2H, d, J=9 Hz)	7.09 (2H, d, J=9 Hz)

Run at 89.55 MHz in pyridine-d_5 solution.

(VIIIb) through an unisolable enol intermediate. In the ^{1}H-NMR spectrum of VIIIb, the singlet signal of the vinylogous methyl proton changed into a doublet and was shifted upfield to δ 1.30 (Δ −0.56 ppm) as compared with that of VIII, while in the ^{13}C-NMR spectrum of VIIIb the olefinic signals of C-3 and C-4 (δ 151.3, 111.4) observed in VIII were absent and a new carbonyl carbon signal appeared at δ 218.1. The signal of H-14a was shifted upfield in the ^{1}H-NMR spectrum of VIIIb, due to the anisotropy of the carbonyl group in the five-



X

VIII: R = –C(=O)–CH(OH)–CH(CH$_3$)$_2$ VIIIa: R = –C(=O)–CH(OH)–CH(CH$_3$)$_2$ VIIIb: R = –C(=O)–CH(OH)–CH(CH$_3$)$_2$

VIIIc: R = H VIId: R = H

IX: R = –C(=O)–CH(OH)–CH(CH$_2$–CH$_3$)CH$_3$

Chart 2

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

Proton No.	VIII	VIIIb	IX
Aglycone moiety			
6	4.58 (1H, t, J=9 Hz)	4.71 (1H, t, J=9 Hz)	4.60 (1H, t, J=9 Hz)
13a	5.58 (1H, d, J=3.1 Hz)	5.69 (1H, d, J=3.1 Hz)	5.58 (1H, d, J=3.1 Hz)
13b	6.25 (1H, d, J=3.3 Hz)	6.34 (1H, d, J=3.3 Hz)	6.27 (1H, d, J=3.3 Hz)
14a	5.47 (1H, br s)	5.03 (1H, br s)	5.49 (1H, br s)
14b	4.97 (1H, br s)	4.80 (1H, br s)	4.99 (1H, br s)
15	1.86 (3H, s)	1.29 (3H, d, J=7 Hz)	1.87 (3H, s)
Ester moiety			
4	1.02 (6H, d, J=7 Hz)	1.13 (6H, d, J=7 Hz)	
5	1.02 (6H, d, J=7 Hz)	1.13 (6H, d, J=7 Hz)	0.83 (3H, t, J=7 Hz)
6			1.02 (3H, d, J=7 Hz)

Run at 89.55 MHz in pyridine-d_5 solution.

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

Carbon No.	VIII	VIIIb	IX	Carbon No.	VIII	VIIIb	IX
Aglycone moiety				Sugar moiety			
1	42.4	40.0	42.5	1	101.6 (161 Hz)		101.7 (156 Hz)
2	35.4	44.5	35.5	2	74.8		74.8
3	151.3	218.1	151.3	3	78.3		78.3
4	111.4	47.2	111.5	4	71.4		71.4
5	53.9	50.7	53.9	5	78.3		78.3
6	80.7	81.8	80.7	6	62.4		62.4
7	48.3	47.2	48.3	Ester moiety			
8	68.2	69.0	68.3	1	174.0	174.2	174.1
9	42.2	43.8	42.5	2	75.9	74.9	75.5
10	143.6	143.4	143.7	3	32.3	32.1	39.0
11	136.0	134.5	136.0	4	17.0	15.9	24.3
12	169.1	168.6	169.1	5	19.3	19.2	11.5
13	121.1	122.7	121.1	6			15.8
14	116.8	116.4	116.8				
15	11.5	14.4	11.5				

Run at 22.5 MHz VIII, IX in pyridine-d_5 and VIIIb in $CDCl_3$.

membered ring. These data indicate that VIIIb, and accordingly VIII, are 1,5-*cis* fused guaianolides.[8)]

Saponification of VIII with alkali gave VIIIc and α-hydroxyisovaleric acid. Acid hydrolysis of VIII gave glucose. The glycosidic linkage was determined to be β by the coupling constant of the anomeric carbon signal ($J_{C_1\text{-}H_1} = 161$ Hz) in the ^{13}C-NMR spectrum of VIII.[9)]

Saponification of VIIIb with alkali gave VIIId and α-hydroxyisovaleric acid, of which the former was shown to be identical with 8-epigrosheimin, which had been isolated from *Crepis virens*,[10)] by comparing the ^{1}H-NMR spectra. These results led us to assign the structure VIII to ixerin S.

Ixerin T (IX), $[\alpha]_D^{19}$ +12.8° has the molecular formula $C_{27}H_{38}O_{11} \cdot 1/2\,H_2O$. Saponification of IX with alkali gave VIIIc and α-hydroxy-β-methyl-*n*-valeric acid. Acid hydrolysis of IX gave glucose. These results led to the structure IX for ixerin T.

Compound X, $C_{21}H_{28}O_9 \cdot H_2O$, $[\alpha]_D^{20}$ −5.7°, was shown to be identical with macrocriniside A[3)] by comparisons of the IR, ^{1}H- and ^{13}C-NMR spectra.

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. Circular dichroism (CD) spectra were recorded with a JASCO J-20A automatic recording spectropolarimeter. IR spectra were taken on a JASCO A-202 infrared spectrophotometer and UV spectra on a Shimadzu UV-360 recording spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL FX-90Q (89.55 and 22.5 MHz, respectively) and JEOL 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-4CM gas chromatograph. HPLC was run on a Kyowa Seimitsu model K 880 instrument.

Isolation——Air-dried whole plants (3.5 kg) of *I. stolonifera* 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, successively. The *n*-butanol-soluble fraction (62 g) was chromatographed on a polyamide (200 g) column with water as an eluent to give Fr. 1 (30 g) and Fr. 2 (1.7 g). Further elution with methanol gave the methanol eluate (14 g). Fraction 1 was rechromatographed on a silica gel column with chloroform–methanol (9 : 1) as an eluent to give ixerins M, N and S, 8-epidesacylcynaropicrin-glucoside and macrocliniside A. Fraction 2 was rechromatographed on a silica gel column with chloroform–methanol (97 : 3) as an eluent to give ixerins O, P, Q and R.

8-Epidesacylcynaropicrin-glucoside (I)——Amorphous powder (150 mg), $[\alpha]_D^{26}$ −31.7° ($c = 1.00$, methanol). *Anal.* Calcd for $C_{21}H_{28}O_9 \cdot 1/2\,H_2O$: C, 58.19; H, 6.74. Found: C, 57.91; H, 6.60. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1665, 1640. NMR (pyridine-d_5): Tables I, II. CD ($c = 2.47 \times 10^{-3}$, H_2O) [θ] (nm): −317 (258).

Ixerin M (II)——Amorphous powder (890 mg), $[\alpha]_D^{19}$ −40.6° ($c = 1.29$, methanol). *Anal.* Calcd for $C_{26}H_{36}O_{11} \cdot 1/2\,H_2O$: C, 58.53; H, 6.99. Found: C, 58.61; H, 6.99. IR ν_{max}^{KBr} cm^{-1}: 3550, 1760, 1740, 1660, 1640. NMR (pyridine-d_5): Tables I, II. CD ($c = 2.15 \times 10^{-3}$, methanol) [θ] (nm): −1567 (275).

Ixerin N (III)——Amorphous powder (1.34 g), $[\alpha]_D^{19}$ −43.5° ($c = 1.00$, methanol). *Anal.* Calcd for $C_{27}H_{38}O_{11} \cdot H_2O$: C, 58.26; H, 7.24. Found: C, 58.42; H, 7.09. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1740, 1660, 1640. NMR (pyridine-d_5): Tables I, II. CD ($c = 2.56 \times 10^{-3}$, methanol) [θ] (nm): −1500 (258).

Ixerin O (IV)——Amorphous powder (80 mg), $[\alpha]_D^{26}$ −34.1° ($c = 2.14$, methanol). *Anal.* Calcd for $C_{34}H_{42}O_{13} \cdot H_2O$: C, 60.35; H, 6.55. Found: C, 60.11; H, 6.29. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1740, 1720, 1688, 1642, 1618, 1600, 1520. UV λ_{max}^{MeOH} nm (log ε): 277.5 (3.22). NMR (pyridine-d_5): Tables III, IV.

Ixerin P (V)——Amorphous powder (60 mg), $[\alpha]_D^{28}$ −23.2° ($c = 0.32$, methanol). *Anal.* Calcd for $C_{34}H_{42}O_{13} \cdot 1/2\,H_2O$: C, 61.16; H, 6.49. Found: C, 61.06; H, 6.49. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1740, 1725, 1670, 1645, 1620, 1600, 1520. UV λ_{max}^{MeOH} nm (log ε): 277.5 (3.41). NMR (pyridine-d_5): Tables III, IV.

Ixerin Q (VI)——Amorphous powder (70 mg), $[\alpha]_D^{26}$ −28.6° ($c = 1.26$, methanol). *Anal.* Calcd for $C_{35}H_{44}O_{13} \cdot H_2O$: C, 60.86; H, 6.71. Found: C, 60.96; H, 6.63. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1722, 1662, 1642, 1620, 1600, 1520. UV λ_{max}^{MeOH} nm (log ε): 277.5 (3.46). NMR (pyridine-d_5): Tables III, IV.

Ixerin R (VII)——Amorphous powder (50 mg), $[\alpha]_D^{23}$ −24.4° ($c = 1.19$, methanol). *Anal.* Calcd for $C_{35}H_{44}O_{13} \cdot H_2O$: C, 60.86; H, 6.71. Found: C, 60.98; H, 6.72. IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1740, 1720, 1670, 1660, 1640, 1620, 1520. UV λ_{max}^{MeOH} nm (log ε): 277.5 (3.25). NMR (pyridine-d_5): Tables III, IV.

Ixerin S (VIII)——Amorphous powder (130 mg), $[\alpha]_D^{19}$ +11.6° ($c = 0.79$, methanol), Anal. Calcd for $C_{26}H_{36}O_{11} \cdot 1/2\,H_2O$: C, 58.53; H, 6.99. Found: C, 58.45; H, 6.83. IR ν_{max}^{KBr} cm^{-1}: 3420, 1760, 1730, 1660, 1640. NMR (pyridine-d_5): Tables III, IV. CD ($c = 2.28 \times 10^{-3}$, methanol) [θ] (nm): −819 (260).

Ixerin T (IX)——Amorphous powder (430 mg), $[\alpha]_D^{19}$ +12.8° ($c=0.80$, methanol). *Anal.* Calcd for $C_{27}H_{38}O_{11}\cdot 1/2\,H_2O$: C, 59.22; H, 7.18. Found: C, 59.40; H, 7.35. IR ν_{max}^{KBr} cm^{-1}: 3430, 1760, 1730, 1660, 1640. NMR (pyridine-d_5): Tables III, IV. CD ($c=2.13\times10^{-3}$, methanol) [θ](nm): −334 (265).

Macrocliniside A (X)——Amorphous powder (120 mg), $[\alpha]_D^{20}$ −5.7° ($c=0.44$, methanol). *Anal.* Calcd for $C_{21}H_{28}O_9\cdot H_2O$: C, 57.01; H, 6.83. Found: C, 57.21; H, 6.66. IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1660. ^{1}H-NMR (pyridine-d_5) δ: 4.98 (1H, d, $J=7.4$ Hz, H-1 of glucose), 5.14 (2H, br s, H_2-14), 5.44 (1H, d, $J=3.1$ Hz, H-13a), 5.51 (1H, br s, H-15b), 5.89 (1H, br s, H-15a), 6.23 (1H, d, $J=3.3$ Hz, H-13b). ^{13}C-NMR (pyridine-d_5) δ: 37.0 (C-7), 37.4 (C-2), 39.8 (C-8), 41.6 (C-1), 49.5 (C-5), 63.0 (C-6 of glucose), 71.8 (C-4 of glucose), 72.3 (C-9), 75.2 (C-2 of glucose), 78.0 (C-5 of glucose), 78.5 (C-3 of glucose), 80.7 (C-3), 84.4 (C-6), 104.2 (C-1 of glucose), 111.2 (C-14), 112.2 (C-15), 118.9 (C-13), 141.1 (C-11), 150.9 (C-4), 153.2 (C-10), 170.0 (C-12).

Acetylation of 8-Epidesacylcynaropicrin-glucoside (I)——8-Epidesacylcynaropicrin-glucoside (I) (40 mg) was dissolved in pyridine and acetic anhydride (each 0.5 ml), and the reaction mixture was left overnight at room temperature. The reagents were evaporated off *in vacuo* and the residue was recrystallized from methanol to give a pentaacetate (Ib) (30 mg), mp 171.0—175.0 °C. IR ν_{max}^{KBr} cm^{-1}: 1750, 1640, 1375, 1230, 1065, 1035, 950, 915. ^{1}H-NMR ($CDCl_3$) δ: 2.00, 2.03, 2.04, 2.05, 2.08 (each 3H, s, $OCOCH_3$), 4.46 (1H, t, $J=9$ Hz, H-6), 4.71 (1H, d, $J=7$ Hz, H-1 of glucose), 5.50 (1H, d, $J=3.1$ Hz, H-13a), 6.29 (1H, d, $J=3.3$ Hz, H-13b).

Acetylation of Ixerin M (II)——Ixerin M (II) (50 mg) was acetylated in the same way as I, to give a pentaacetate (IIa) (40 mg), mp 167.0—168.5 °C. *Anal.* Calcd for $C_{36}H_{46}O_{16}$: C, 58.85; H, 6.31. Found: C, 58.57; H, 6.16. IR ν_{max}^{KBr} cm^{-1}: 1770, 1755, 1665, 1640, 1370, 1320, 1230, 1135, 1065, 1035. ^{1}H-NMR ($CDCl_3$) δ: 2.03, 2.04, 2.06, 2.10, 2.14 (each 3H, s, $OCOCH_3$). ^{13}C-NMR: Table I.

Acetylation of Ixerin N (III)——Ixerin N (III) (50 mg) was acetylated in the same way as I, and gave a pentaacetate (IIIa) (40 mg), mp 148.5—149.5 °C. *Anal.* Calcd for $C_{37}H_{48}O_{16}$: C, 59.35; H, 6.46. Found: C, 59.09; H, 6.44. IR ν_{max}^{KBr} cm^{-1}: 1765, 1750, 1668, 1642, 1370, 1320, 1230, 1140, 1070, 1040. ^{1}H-NMR ($CDCl_3$) δ: 2.01, 2.03, 2.04, 2.08, 2.09 (each 3H, s, $OCOCH_3$). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 8-Epidesacylcynaropicrin-glucoside (I)——8-Epidesacylcynaropicrin-glucoside (30 mg) was dissolved in water (3 ml) and the solution was treated with crude hesperidinase (10 mg) for 3 h at 35 °C with stirring. The solution was passed through an Amberlite XAD-2 column, and the eluate with methanol was purified on a silica gel column to give an aglycone (Ia) (23 mg). $[\alpha]_D^{24}$ −17.2° ($c=0.29$, pyridine). *Anal.* Calcd for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.58; H, 6.91. ^{1}H-NMR (400 MHz) ($CDCl_3$–CD_3OD, 1 : 1) δ: 1.76 (1H, ddd, $J=13$ Hz, $J=9.5$ Hz, $J=9$ Hz, H-2), 2.21 (1H, ddd, $J=13$ Hz, $J=7.5$ Hz, $J=7.5$ Hz, H-2), 2.36 (1H, dd, $J=13.5$ Hz, $J=6$ Hz, H-9), 2.59 (1H, dd, $J=13.5$ Hz, $J=6$ Hz, H-9), 2.79 (1H, dd, $J=10$ Hz, $J=9$ Hz, H-5), 2.89 (1H, ddd, $J=9.5$ Hz, $J=9.5$ Hz, $J=7.5$ Hz, H-1), 3.00 (1H, ddd, $J=9$ Hz, $J=3.3$ Hz, $J=3$ Hz, H-7), 4.30 (1H, ddd, $J=6$ Hz, $J=6$ Hz, $J=3$ Hz, H-8), 4.51 (1H, dddd, $J=9$ Hz, $J=7.5$ Hz, $J=1$ Hz, $J=1$ Hz, H-3), 4.57 (1H, dd, $J=10$ Hz, $J=9$ Hz, H-6), 4.95 (1H, br s, H-14b), 5.06 (1H, br s, H-14a), 5.33 (1H, br s, H-15b), 5.43 (1H, br s, H-15a), 5.64 (1H, d, $J=3.3$ Hz, H-13a), 6.35 (1H, d, $J=3.3$ Hz, H-13b).

Enzymatic Hydrolysis of Ixerin S (VIII)——Ixerin S (VIII) (11 mg) was dissolved in water (2 ml), and the solution was treated with crude hesperidinase (5 mg) for 2 h at 35 °C with stirring. The solution was passed through an Amberlite XAD-2 column, and the fraction eluted with methanol was purified on a silica gel column to give an aglycone (VIIIb). ^{1}H-NMR and ^{13}C-NMR: Tables III, IV.

Partial Hydrolysis of Ixerin O (IV) and P (V)——About 0.5 mg of IV or V was treated with 1% barium hydroxide (5 drops) under a nitrogen atmosphere for 1 h at room temperature. The reaction mixture was acidified with hydrochloric acid and concentrated. The residue gave *p*-hydroxyphenylacetic acid and II, which were identified by HPLC, in both cases. Conditions: column, TSK gel LS-410AK 4 mm × 30 cm: solvent, acetonitrile–water (3 : 7); flow rate, 1.0 ml/min; detector, UV 220 nm; t_R 2.8 min (*p*-hydroxyphenylacetic acid), 6.5 min (ixerin M).

Partial Hydrolysis of Ixerin Q (VI) and R (VII)——About 0.5 mg of VI or VII was treated with 1% barium hydroxide (5 drops) under a nitrogen atmosphere for 1 h at room temperature. The reaction mixture was acidified with hydrochloric acid and extracted with ethyl acetate. After concentration of the organic layer the products were identified as *p*-hydroxyphenylacetic acid and III by HPLC in both cases. Conditions: column, TSK gel LS-410AK 4 mm × 30 cm: solvent, acetonitrile–water (3 : 7); flow rate, 1.0 ml/min; detector, UV 220 nm; t_R 2.8 min (*p*-hydroxyphenylacetic acid), 6.9 min (ixerin N).

Saponification of Ixerin M (II)——Ixerin M (II) (45 mg) was treated with 2% sodium hydroxide (3 ml) under a nitrogen atmosphere for 1.5 h at room temperature. The mixture was acidified with hydrochloric acid and extracted with ether. The ether layer contained α-hydroxyisovaleric acid. The water layer was passed through an Amberlite XAD-2 column, and the fraction eluted with methanol was purified on a silica gel column to give 8-epidesacylcynaropicrin-glucoside (I) (19 mg). α-Hydroxyisovaleric acid was made into *p*-bromophenacyl ester and it was shown to be identical with an authentic sample by HPLC. Conditions: column, TSK gel LS-410AK 4 mm × 30 cm; solvent, acetonitrile–water (35 : 65); flow rate, 1.1 ml/min; detector, UV 254 nm; t_R 5.8 min.

Saponification of Ixerin N (III)——III (91 mg) was saponified in the same way as II, to give I (40 mg) and α-hydroxy-β-methyl-*n*-valeric acid, whose *p*-bromophenacylester was identified by HPLC comparison with an authentic sample. HPLC conditions: column, TSK gel LS-410AK 4 mm × 30 cm; solvent, acetonitrile–water (35 : 65);

flow rate, 1.1 ml/min; detector UV 254 nm; t_R 6.8 min.

Saponification of Ixerin S (VIII)——VIII (12 mg) was saponified in the same way as II, to give VIIIc (9 mg) and α-hydroxyisovaleric acid. VIIIc, colorless plates from methanol–ethyl acetate, mp 225.0—227.0 °C. *Anal.* Calcd for $C_{21}H_{28}O_9$: C, 59.42; H, 6.65. Found: C, 59.16; H, 6.40. ^{1}H-NMR (pyridine-d_5) δ: 1.92 (3H, s, H_3-15), 4.86 (1H, t, J= 9 Hz, H-6), 5.08 (1H, br s, H-14b), 5.30 (1H, d, J=7.2 Hz, H-1 of glucose), 5.44 (1H, br s, H-14a), 5.68 (1H, d, J= 3.1 Hz, H-13a), 6.48 (1H, d, J=3.3 Hz, H-13b). ^{13}C-NMR (pyridine-d_5) δ: 11.7 (C-15), 35.7 (C-2), 43.2 (C-1), 45.7 (C-9), 50.1 (C-7), 54.2 (C-5), 62.5 (C-6 of glucose), 65.9 (C-8), 71.4 (C-4 of glucose), 74.9 (C-2 of glucose), 78.4 (C-3 of glucose and C-5 of glucose), 80.4 (C-6), 101.8 (C-1 of glucose), 112.4 (C-4), 115.8 (C-14), 120.8 (C-13), 137.8 (C-11), 144.9 (C-10), 151.3 (C-3), 170.3 (C-12).

Saponification of Ixerin T (IX)——IX (41 mg) was saponified in the same way as II, to give VIIIc (25 mg) and α-hydroxy-β-methyl-*n*-valeric acid.

Saponification of VIIIb——VIIIb (14 mg) was saponified in the same way as II, to give VIIId (7 mg) and α-hydroxyisovaleric acid.

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, 3 mm × 2 m; column temperature, 230 °C; carrier gas, N_2; flow rate, 60 ml/min; t_R 8.9 min.

Synthesis of *p*-Bromophenacyl Ester[11]——Standard samples of α-hydroxyisovaleric acid and α-hydroxy-β-methyl-*n*-valeric acid were prepared from valine and isoleucine by the Van Slyk method. Each acid was treated with *p*-bromophenacyl bromide in water (pH 5) containing a small amount of ethanol for 1 h under reflux, and the product was purified by TLC.

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