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Sesquiterpene Lactones from *Ixeris tamagawaensis* KITAM. II¹⁾

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Three new guaianolide glucosides, ixerins D, E and F, and a new melampolide glucoside, ixerin G, have been isolated from the polar fraction of *Ixeris tamagawaensis* KITAM. Their structures were elucidated on the basis of spectral data and several chemical transformations.

Keywords—*Ixeris tamagawaensis*; Compositae; sesquiterpene lactone; glycoside; ixerin D; ixerin E; ixerin F

This study is a continuation of our work on the constituents of *Ixeris tamagawaensis* KITAM. (Compositae), which produces cytotoxic and antitumor sesquiterpene lactones, and from which we have previously isolated four sesquiterpenes, ixerins A, B and C and 8-desoxyurospermal A.¹⁾ In the present paper, we describe the isolation of four new sesquiterpene lactones, obtained in rather small quantities. One of these is a melampolide, ixerin G. The other three substances are guaianolides, ixerins D, E and F.

We will discuss first the structure elucidation of ixerin D (I), which has the molecular formula $C_{21}H_{30}O_9$, mp 230—231 °C. Its infrared (IR) spectrum showed absorptions at 3450 cm^{-1} (hydroxyl) and 1773 cm^{-1} (lactone), and the proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectrum showed two doublet signals [δ 5.35 ($J=3.0\text{ Hz}$), δ 6.20 ($J=3.1\text{ Hz}$)] which are characteristic of the exocyclic methylene protons of an α -methylene- γ -lactone group. Decoupling experiments showed that the olefinic protons at δ 5.42 and δ 5.72 were coupled with H-3 and H-5, respectively. In the $^1\text{H-NMR}$ spectrum, the anomeric proton appeared as a doublet centered at δ 5.10 ($J=7\text{ Hz}$). On the other hand, in the $^{13}\text{C-nuclear}$ magnetic resonance ($^{13}\text{C-NMR}$) spectrum, in addition to the signals of glucopyranose attached to secondary alcohol, fifteen signals were assignable to the aglycone. Thus, we deduced that this compound is a sesquiterpene glucoside having the structure I. The application of circular dichroism (CD) spectroscopy has been of great value in the determination of the stereochemistry of many sesquiterpene lactones.²⁾ The CD spectral data of I led us to the conclusion that the 6,7-fused lactone was *trans*, and this was confirmed by the coupling constant.³⁾

Acetylation of I with acetic anhydride in pyridine at room temperature afforded the tetraacetate (II), and dehydration with POCl_3 gave III, which was identical with glucozalanin C tetraacetate.⁴⁾

The stereochemistry of the C-10 hydroxyl group was determined as follows. The enzymatic hydrolysis of ixerin D gave the aglycone (IV). The acetylation of IV with acetic anhydride in pyridine at room temperature afforded the monoacetate (V) and at 100 °C gave the diacetate (VI). When the $^1\text{H-NMR}$ spectra of V and VI were compared, it became apparent that the chemical shifts of H-5 and H-7 of VI exhibited upfield shifts of 0.06 and 0.25 ppm, respectively. This kind of effect is observed only when the hydroxyl group is close to H-5 and H-7, so that the C-10 hydroxyl group must be α -oriented. Acid hydrolysis of ixerin D gave glucose.

Ixerin E (VII) has the molecular formula $C_{21}H_{32}O_9$, mp 214.5—216 °C. The IR and $^1\text{H-}$

TABLE I. ^1H -NMR Data for IV, V, VI in CDCl_3 (400 MHz)

	IV	V	VI
1	2.23 (m)	2.25 (m)	2.44 (m)
2	1.96 (m)	2.25 (m)	2.44 (m)
3	2.32 (m)	2.42 (m)	2.71 (m)
5	4.55 (br t, $J=6.5$ Hz)	5.53 (br t, $J=6.8$ Hz)	5.53 (br t, $J=7.5$ Hz)
6	2.84 (br t, $J=9.3$ Hz)	2.86 (br t, $J=9.2$ Hz)	2.80 (t, $J=9.0$ Hz)
7	4.24 (t, $J=10.1$ Hz)	4.15 (t, $J=10.1$ Hz)	4.13 (dd, $J=9.5, 9.6$ Hz)
8	3.15 (m)	3.21 (m)	2.96 (m)
9	1.45—1.75 (m)	1.50—1.95 (m)	1.53—1.65 (m)
13a	1.45—1.75 (m)	1.50—1.95 (m)	1.53—1.65 (m)
13b	5.45 (d, $J=3.3$ Hz)	5.46 (d, $J=3.2$ Hz)	5.45 (d, $J=3.4$ Hz)
14	6.18 (d, $J=3.8$ Hz)	6.18 (d, $J=3.5$ Hz)	6.19 (d, $J=3.5$ Hz)
15	1.24 (s)	1.24 (s)	1.52 (s)
Ac	5.28 (br s)	5.29 (br s)	5.36 (br s)
	5.35 (br s)	5.41 (br s)	5.44 (br s)
		2.08 (s)	2.04, 2.07 (s)

NMR spectra were similar to those of ixerin D, but there was a methyl doublet at δ 1.18 ($J=7$ Hz) instead of the usual two doublets, and the γ -lactone carbonyl resonated at δ 178.4 in the ^{13}C -NMR spectrum, indicating an 11,13-dihydro lactone. Thus, we considered the structure to be VII. The reduction of ixerin D (I) with NaBH_4 afforded only one product (VII), which was identical with ixerin E based on a comparison of the IR and ^1H -NMR spectra.

When an α -methylene- γ -lactone is reduced with NaBH_4 , the reduction product generally has an α -oriented methyl group.^{4,5)} The methyl group of VIIa exhibited an upfield shift of 0.22 ppm in benzene- d_6 relative to chloroform- d_1 solution, and from Narayanan's rule the methyl group should be α -oriented.⁶⁾ Acid hydrolysis of ixerin E gave glucose.

Ixin F (VIII) has the molecular formula $\text{C}_{21}\text{H}_{30}\text{O}_9 \cdot 1/2\text{H}_2\text{O}$. The IR spectrum of ixerin F showed strong absorptions at 3380 cm^{-1} (hydroxyl) and 1750 cm^{-1} (lactone). Enzymatic hydrolysis of ixerin F (VIII) afforded an aglycone (IX) and its ^1H -NMR spectrum showed a

doublet methyl signal at δ 1.10 ($J=7$ Hz) instead of the two doublets characteristic of an exocyclic methylene in a γ -lactone group. The remaining part of the structure was evident from spin-decoupling experiments on IX. The H-15 olefinic proton signals were seen as triplets ($J=1$ Hz) at δ 5.32 and δ 5.40 showing long-range coupling with H-3 [δ 4.65 (m)] and H-5 [δ 2.90 (tt, $J=9.5, 1$ Hz)]. An olefinic proton signal at δ 5.10 (2H, br s) was assigned to H-14 and also had long-range coupling with H-1 (δ 3.30 m). Irradiation at this proton converted the H-5 triple triplet to a double triplet in addition to sharpening the H-14 singlet signal. Further, a triplet at δ 4.00 ($J=9$ Hz) is characteristic of a guaianolide with a 6,7-fused lactone. The signals in the region of δ 4.60 to δ 4.70 corresponded to 2H; one of these hydrogens was unambiguously H-3, while the other proton is attached to the α -carbon carrying a secondary alcohol. Acetylation of IX supported the presence of two hydroxyl groups. One hydroxyl group was attached to C-3, but the possibility of 2,3-diol system was ruled out by spin-decoupling experiments, so that the other hydroxyl group must be attached to C-9 or C-8. On the basis of a comparison of the ^{13}C -NMR spectrum of VIII with that of 11,13 α -dihydroglucozaluzanin C, which was previously prepared in our laboratory, the remaining hydroxyl group was attached to C-9. That is to say, the C-9 carbon signal at δ 72.9 in ixerin F showed a downfield shift of 36.9 ppm and the β -carbons, C-10 and C-8, exhibited downfield shifts of 3.9 and 8.4 ppm, respectively. Furthermore, the γ -carbons C-1, C-7 and C-14 displayed upfield shifts of 2.4, 5.7 and 2.4 ppm, respectively. The above conclusion was confirmed by the existence of allylic coupling between H-9 and H-14. Irradiation at the H-9 signal converted the broad singlet of H-14 into a sharp singlet. Subsequently, in order to determine the stereochemistry of the C-9 hydroxyl group we compared the ^1H -NMR spectrum of IX with that of X. The signals of H-1 and H-7 of X showed upfield shifts of 0.2 and 0.1 ppm, respectively. These shifts require that the hydroxyl group has the same spatial direction as H-7 and H-1. If it is assumed that all naturally occurring sesquiterpene lactones have α -oriented H-7 on the basis of biosynthetic considerations, the C-9 hydroxyl group and H-1 are α -oriented in ixerin F (VIII). The configuration of C-5 was determined as follows. The ^1H -NMR chemical shift of H-14a (Chart 3) showed an upfield shift in XI, obtained by oxidation with CrO_3 , compared with that of IX and no upfield shift was observed in the acetylated derivative X. Thus, H-14a is more shielded than in other guaianolides. This is due to the anisotropy of the carbonyl group in the five-membered ring. The H-14a olefinic proton is

Chart 2

Chart 3

TABLE II. ^1H -NMR Data for IX, X, XI in CDCl_3 (90 MHz)

	IX	X	XI
1	3.30 (m)	3.10 (m)	3.80 (m)
2	1.5—2.6 (m)	1.6—2.6 (m)	1.5—2.9 (m)
3	4.65 (m)	5.59 (br t, $J=6.5$ Hz)	—
5	2.90 (tt, $J=9.5, 1$ Hz)	2.83 (tt, $J=9.5, 1$ Hz)	3.18 (tt, $J=9.5, 1$ Hz)
6	4.00 (t, $J=9$ Hz)	3.98 (t, $J=9$ Hz)	3.95 (t, $J=9$ Hz)
7	1.5—2.6 (m)	1.6—2.6 (m)	1.5—2.9 (m)
8	1.5—2.6 (m)	1.6—2.6 (m)	1.5—2.9 (m)
9	4.65 (m)	5.55 (br t, $J=4$ Hz)	4.60 (br s)
11	1.5—2.6 (m)	1.6—2.6 (m)	1.5—2.9 (m)
13	1.30 (d, $J=7$ Hz)	1.23 (d, $J=7$ Hz)	1.30 (d, $J=7$ Hz)
14	5.10 (s)	5.10 (br s) 5.18 (br s)	4.62 (br s) 5.05 (br s)
15	5.32 (t, $J=1$ Hz) 5.40 (t, $J=1$ Hz)	5.29 (t, $J=1$ Hz) 5.42 (t, $J=1$ Hz)	5.75 (d, $J=3$ Hz) 6.25 (d, $J=3$ Hz)
Ac		2.10×2 (s)	

right above the $\text{C}=\text{O}$ double bond plane. However, in a 1,5-*trans* fused guaianolide, the H-14a proton cannot adopt such a position. The most probable conformation of ixerin F, deduced from these data and from analysis of the Dreiding stereomodel, is shown in Chart 3. The stereochemistry of C-13 and the γ -lactone ring was determined from the results described below. The NaBH_4 reduction of XV, which was previously isolated in our laboratory and has a 6,7-*trans* fused lactone ring ($J_{7-13a}=3.3$ Hz, $J_{7-13b}=3.1$ Hz), gave dihydro derivative, which was identified as ixerin F (VIII) (^1H -NMR and IR). The stereochemistry of C-3 is still unknown. However, the chemical shifts of C-2, C-3, C-4 and C-5 were nearly the same as those of other guaianolides with a β -oriented hydroxyl group, so the hydroxyl group of ixerin F (VIII) may be β -oriented. Acid hydrolysis of ixerin F gave glucose.

Ixin G (XII) has the molecular formula $\text{C}_{29}\text{H}_{34}\text{O}_{11} \cdot 1/2\text{H}_2\text{O}$ and its ultraviolet (UV) spectrum showed maximum absorptions at 227.5 and 277.5 nm. Its IR spectrum showed absorptions at 3420 cm^{-1} (hydroxyl), 1750 cm^{-1} (lactone) and 1742 cm^{-1} (aldehyde). The ^1H -NMR spectrum was nearly the same as that of ixerin C (XIII), which had previously been isolated from this plant in our laboratory.¹⁾

Saponification with alkali gave *p*-hydroxyphenylacetic acid and ixerin B (XIV), which had also been isolated previously, so that ixerin G has *p*-hydroxyphenylacetic acid esterified at the glucose moiety of ixerin B. In order to determine the structure, we compared the ^{13}C -NMR spectrum with that of ixerin B. The signals due to glucose exhibited upfield shifts of 3.2 ppm at C-3 and 3.3 ppm at C-1, but a downfield shift of 1.1 ppm at C-2. From these results, we concluded that the *p*-hydroxyphenylacetic acid moiety is bound at C-2 of the

TABLE III. ^{13}C -NMR Data for I, VII, VIII, 11,13-Dihydroglucozaluzanin C

	I	VII	VIII	11,13-Dihydro- glucozaluzanin C
Aglycone moiety				
1	50.3 ^{a)}	48.7 ^{c)}	41.8	44.2 ^{f)}
2	35.4 ^{b)}	35.3 ^{d)}	37.3	37.8
3	79.8	79.9	80.6	80.5
4	150.7	151.5	151.2	150.7 ^{h)}
5	50.7 ^{a)}	49.7 ^{c)}	49.4	50.2 ^{g)}
6	81.9	82.6	83.9	83.4
7	44.5	39.0	36.5	42.2 ^{f)}
8	24.9	26.4	40.7	32.3
9	35.5 ^{b)}	35.2 ^{d)}	72.9	36.0
10	73.4	73.6	153.5	149.6 ^{h)}
11	142.8	43.2	45.3	50.0 ^{g)}
12	170.1	178.5	178.4	178.2
13	118.2	13.3	13.2	13.3
14	30.3	28.1	110.8 ^{e)}	113.2
15	112.9	110.6	111.6 ^{e)}	112.0
Glucose moiety				
1	103.6	104.0	104.3	104.1
2	75.2	75.3	75.2	75.2
3	78.4	78.5	78.4	78.5
4	71.9	72.0	71.8	71.8
5	78.1	78.2	78.0	78.1
6	63.0	63.0	62.9	63.0

a—h) May be interchanged in each column (measured in pyridine- d_5).

Chart 4

glucose moiety of ixerin B. Acid hydrolysis of ixerin G afforded glucose.

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 CD was determined with a JASCO J-400 X spectropolarimeter. IR spectra were run on a JASCO A 202 grating infrared spectrophotometer, UV spectra on a Shimadzu UV-360 recording spectrophotometer and mass spectra (MS) on a JEOL JMS-D/100 mass spectrometer. NMR spectra were recorded on JEOL JNM GX-400 and JEOL FX-90 Q spectrometers (^1H -NMR: 399.65 and 89.55 MHz, ^{13}C -NMR: 22.5 MHz); chemical shifts were given in ppm with tetramethylsilane as an internal standard. Field desorption (FD)-MS were measured on a JEOL JMS-D/300, with an MS-FD/03 ion source.

Isolation—Whole plants of *Ixeris tamagawaensis* (7.2 kg) were extracted with methanol under reflux. The

TABLE IV. ^{13}C -NMR Data for XII, XIV in Pyridine- d_5

	XII	XIV
Aglycone moiety		
1	153.3	153.4
2	27.3	27.1
3	33.3	33.5
4	138.1	138.4
5	129.7	129.7
6	79.5	79.4
7	46.2	46.1
8	22.2	22.1
9	24.3	24.1
10	145.0	145.0
11	140.6	140.5
12	170.1	170.1
13	118.2	118.2
14	195.9	196.1
15	67.4	67.8
Glucose moiety		
1	101.7	105.0
2	76.1	75.0
3	75.3	78.5
4	71.9	71.6
5	78.7	78.5
6	62.6	62.8
<i>p</i> -Hydroxyphenylacetic acid moiety		
α	171.2	
β	40.9	
1	125.2	
2	116.3	
3	131.0	
4	157.9	
5	131.0	
6	116.3	

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 to give sesquiterpenes.

Ixerin D (I)—Colorless needles (30 mg), mp 230–231 °C (MeOH). $[\alpha]_D^{19}$: -32.7° ($c=0.51$, MeOH). *Anal.* Calcd for $\text{C}_{21}\text{H}_{30}\text{O}_9$: C, 59.14; H, 7.09. Found: C, 58.89; H, 7.20. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450, 3300, 1773. ^1H -NMR (pyridine- d_5) δ : 1.32 (3H, s, H-14), 2.95 (1H, br t, $J=8.5$ Hz, H-5), 3.35 (1H, m, H-7), 4.89 (1H, br t, $J=7$ Hz, H-3), 5.10 (1H, d, $J=7$ Hz, anomeric), 5.35 (1H, d, $J=3.0$ Hz, H-13a), 5.42 (1H, br s, H-15), 5.72 (1H, br s, H-15), 6.20 (1H, d, $J=3.1$ Hz, H-13b). CD ($c=3.14 \times 10^{-4}$, MeOH) $[\theta]$ (nm): -4500 (255). FD-MS m/z : 427 ($\text{M}+\text{H}$) $^+$.

Ixerin E (VII)—Colorless needles (11 mg) mp 214.5–216 °C (MeOH- C_6H_6). $[\alpha]_D^{19}$: -12.3° ($c=0.16$, MeOH). *Anal.* Calcd for $\text{C}_{21}\text{H}_{32}\text{O}_9$: C, 58.87; H, 7.52. Found: C, 58.78; H, 7.26. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3500, 3460, 1775. ^1H -NMR (pyridine- d_5) δ : 1.25 (3H, s, H-14), 1.18 (3H, d, $J=7$ Hz, H-13), 2.82 (1H, br t, $J=8.5$ Hz, H-5), 4.82 (1H, br t, $J=7$ Hz, H-3), 4.99 (1H, d, $J=7$ Hz, anomeric-H), 5.34, 5.72 (each 1H, br s, H-15). FD-MS: m/z : 429 ($\text{M}+\text{H}$) $^+$.

Ixerin F (VIII)—Amorphous powder (700 mg). $[\alpha]_D^{19}$: $+1.0^\circ$ ($c=0.48$, MeOH). *Anal.* Calcd for $\text{C}_{21}\text{H}_{30}\text{O}_9 \cdot 1/2\text{H}_2\text{O}$: C, 57.92; H, 7.20. Found: C, 57.66; H, 7.04. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3380, 1750. ^1H -NMR (pyridine- d_5) δ : 1.10 (3H, d, $J=7$ Hz, CH_3), 2.89 (1H, br t, $J=8.5$ Hz, H-5), 4.95 (1H, d, $J=7.5$ Hz, anomeric-H), 5.08 (2H, br s, H-14), 6.45, 6.85 (each 1H, br s, H-15). FD-MS m/z : 427 ($\text{M}+\text{H}$) $^+$.

Ixerin G (XII)—Amorphous powder (5 mg). $[\alpha]_D^{22}$: -15.1° ($c=0.11$, MeOH). *Anal.* Calcd for $\text{C}_{29}\text{H}_{34}\text{O}_{11} \cdot 1/2\text{H}_2\text{O}$: C, 61.38; H, 6.22. Found: C, 61.55; H, 6.15. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3420, 1750, 1742. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 227.5 (4.19), 277.5 (3.37). ^1H -NMR (pyridine- d_5) δ : 3.85 (2H, s, Ar- CH_2), 5.01 (1H, d, $J=8$ Hz, anomeric-H), 5.39 (1H, d, $J=3.3$ Hz, H-13a), 5.61 (1H, t, $J=9.5$ Hz, H-2 of glucose moiety), 6.38 (1H, br t, $J=8$ Hz, H-1), 6.81 (1H, d, $J=3.3$ Hz, H-13b), 7.06 (2H, d, $J=9$ Hz, H-2,6 of ester), 7.44 (2H, d, $J=9$ Hz, H-3, 5 of ester), 9.53 (1H, br s, H-14). FD-MS m/z : 559 ($\text{M}+\text{H}$) $^+$.

Dehydration of I—Ixin D (15 mg) was dissolved in acetic anhydride and pyridine (0.5 ml each) and the solution was left for 30 min at room temperature. The mixture was evaporated *in vacuo*, and 0.5 ml of pyridine and 5 drops of POCl₃ were added to the residue. The mixture was left to stand for 4 h at room temperature, then excess H₂O was added in order to destroy the reagent. The aqueous solution was extracted with ethyl acetate 3 times. The residue (19 mg) from the extract was purified by silica gel column to afford colorless needles (III) (recrystallized from methanol). This product was identical with authentic glucozaluzanin C tetraacetate [mp, ¹H-NMR, IR] mp 159.5—161 °C (lit. 159—160 °C). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1770, 1746.

Enzymatic Hydrolysis of I—Ixin D (100 mg) was dissolved in water (3 ml) and treated with hesperidinase for 3 h at 35 °C with stirring. The solution was extracted with ethyl acetate 3 times and purified on a silica gel column to give an aglycone (IV) (amorphous powder 45 mg). ¹H-NMR: Table I.

Acetylation of IV—IV (20 mg) was dissolved in acetic anhydride and pyridine (2 ml each). The solution was left for 12 h, then concentrated *in vacuo* to give the monoacetate (V) (25 mg). V (10 mg) was dissolved in acetic anhydride and pyridine (1 ml each), and the solution was heated at 100 °C in a water bath for 10 h to give the diacetate (VI). These compounds were purified by silica gel column chromatography (amorphous powders, 8 and 5 mg, respectively). ¹H-NMR: Table I.

Reduction of I—Ixin D (I) (50 mg) was dissolved in methanol (5 ml) and stirred with NaBH₄ (20 mg) for 1 h at room temperature. 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 5 times. Recrystallization from benzene-methanol gave colorless needles (VII) (45 mg) (mp 214.5—216 °C). This product was identical with ixerin E [¹H-NMR, IR, mixed mp]. ¹H-NMR, ¹³C-NMR: ixerin E.

Enzymatic Hydrolysis of VIII—Ixin F (VIII) (240 mg) and hesperidinase (120 mg) were dissolved in water and the solution was stirred for 12 h at 35 °C, then extracted with ethyl acetate 3 times. The residue from the extract was purified by silica gel column chromatography to give the aglycone (IX) (amorphous powder 90 mg). ¹H-NMR: Table II.

Oxidation of IX—IX (20 mg) was dissolved in pyridine and treated with CrO₃-pyridine complex. The mixture was stirred for 8 h at room temperature. After being diluted with water, the reaction mixture was passed through an Amberlite XAD-2 column, which was washed with water. The methanol eluate was purified on a silica gel column chromatography to give the monoketone (XI) (amorphous powder 3 mg). ¹H-NMR: Table II.

Acetylation of IX—IX (5 mg) was dissolved in pyridine and acetic anhydride (1 ml each) and the solution was left for 12 h. The solution was concentrated *in vacuo* to give a residue, which was purified by silica gel column to provide 5 mg of pure X (amorphous powder). ¹H-NMR: Table II.

Saponification of XII—A solution of ixerin F (XII) (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 and *n*-butanol 3 times. The ethyl acetate extract was concentrated to give *p*-hydroxyphenylacetic acid, which was detected by high performance liquid chromatography (HPLC) in comparison with a standard sample. The *n*-butanol extract was concentrated to give ixerin B (XIV), which was identified by thin layer chromatography (TLC). HPLC conditions: Lichrosorb RP-8 column, 4 mm × 25 cm; eluent, H₂O-CH₃CN (75:25); UV detector at 240 nm.

Acid Hydrolysis of Glycosides—A solution of a glycoside (*ca.* 1 mg) in 10% H₂SO₄ (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 NaBH₄ (*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 reagents were evaporated off *in vacuo*. Glucitol acetate was detected by gas chromatography from each glycoside. Conditions: 1.5% OV-17 column, 3 mm × 1 m; column temperature, 230 °C; carrier gas, N₂; *t*_R, 3.8 min.

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