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Lasianthionosides A–C, megastigmane glucosides from leaves of *Lasianthus fordii*

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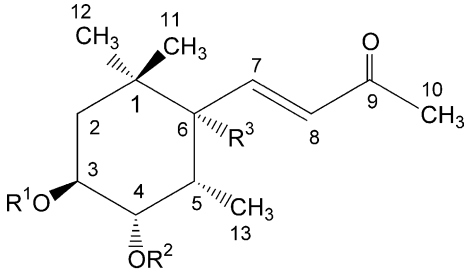
Abstract

From the leaves of *Lasianthus fordii*, three megastigmane glucosides, lasianthionosides A, B and C, were isolated together with the known iridoid glucoside, asperuloside, deacetylasperuloside and methyl deacetyl-asperulosidate, and a megastigmane glucoside, citroside A. The structures have been elucidated based on spectroscopic analyses and/or X-ray crystallographic analysis.

Keywords: *Lasianthus fordii*; Rubiaceae; Lasianthionosides A, B and C; Megastigmane glucoside

1. Introduction

To the best of our knowledge, no reports have appeared on the constituents of *Lasianthus fordii* Hance (Rubiaceae) (Hatusima and Amano, 1994). During the course of our studies on the constituents of the plants grown under a subtropical climate, we examined the glycosidic constituents of the leaves of *L. fordii* and isolated three new megastigmane glucosides, lasianthionosides A (**1**), B (**2**) and C (**3**) together with known iridoid glucosides, asperuloside (Briggs et al., 1963), deacetylasperuloside (Inouye et al., 1969), and methyl deacetylasperulosidate (Inouye et al., 1969), and a known megastigmane glucoside, citroside A (Umehara et al., 1988). This paper describes the isolation and structural elucidation of the new compounds.



(**1**) R^1=H ; R^2=Glc ; R^3=OH
(**2**) R^1=Glc ; R^2=R^3=H
(**3**) R^1=R^3=H ; R^2=Glc
Glc=β-D-glucopyranose

2. Results and discussion

The *n*-BuOH soluble fraction of the methanolic extract of the leaves was separated by combination of Diaion HP-20 and silica gel chromatographies, and finally by HPLC (see Section 3) to give lasianthionosides A (**1**), B (**2**) and C (**3**) in addition to asperuloside, deacetylasperuloside, methyl deacetylasperulosidate and citroside A.

Lasianthionoside A (**1**), $[\alpha]_D$ −76.1° (C_5H_5N) was obtained as colorless needles, mp 209–210 °C, and the molecular formula was determined as $C_{19}H_{32}O_9$ based on its negative ion HR-FAB mass spectrum. It showed

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a UV absorption maximum at 232 nm, indicative of a conjugated enone structure. The ^{13}C NMR spectrum (Table 1) showed, in addition to the signals due to a β-glucopyranosyl moiety, 13 signals which consisted of four methyl groups, a methylene group, a methine group, a quaternary carbon atom, two methine groups having an oxygen atom, a quaternary carbon having an oxygen atom, a disubstituted double bond and a ketone group conjugated with an aforementioned double bond. These data suggested that lasianthionoside A (**1**) is a megastigmane glucoside. From the inspection of the ^1H NMR spectrum (C_5D_5N), the double bond [δ 6.82 and 7.09 (each 1H, *d*, $J = 15.6$ Hz)] was conjugated with an acetyl group [δ 2.28 (3H, s)] and adjacent to a quaternary carbon atom, leading to the structure of the side chain. The structure of the remainder, the molecule was elucidated by analysing the ^1H–^1H COSY spectrum. Starting from methylene protons at C-2, one (δ 2.57) of which showed cross peaks with one (δ 1.54) of the *gem*-dimethyl groups, cross peaks were followed to δ 4.79 (H-3)→δ 4.49 (H-4)→δ 2.90 (H-5)→δ 1.26 (H_3-13). Thus, two secondary carbinyl functional groups are located at C-3 and C-4 and take an axial orientation as judged from the coupling patttern of the proton geminal to these oxygen substituents. The remaining quaternary carbon atom having an oxygen atom was thus assigned as located at C-6. Based on the above discussion, the structure of the aglucone part was elucidated as 3,4,6-trihydroxy-7-megastigmen-9-one. The relative stereochemistry was determined as shown based on the results obtained from precise differential NOE experiments (in Fig. 1). The glycosidic linkage was determined to be located on the oxygen atom at C-4 and had a β-configuration based on cross peaks between the signal at δ 4.79 (H-3) and 6.62 (3-OH) they observed in the ^1H–^1H COSY spectrum, as well as NOE's observed for H-3 and H-4 upon irradiation of the anomeric signal at δ 4.94 (*d*, $J = 7.8$ Hz). Thus, the structure of lasianthionoside A was elucidated as **1**. The absolute stereochemistry will be discussed later.

Table 1
^{13}C NMR spectroscopic data of lasianthionosides A (**1**), B (**2**) and C (**3**) (CD_3OD)

Carbon	1	2	3
1	39.5	34.7	34.5
2	38.1	40.9	41.8
3	68.0	79.2	68.5
4	82.2	72.0	80.7
5	32.7	31.4	30.7
6	80.6	52.0	52.6
7	153.1	152.7	152.8
8	131.3	134.0	134.2
9	201.1	200.9	201.1
10	27.3	27.0	26.8
11	27.6	23.8	24.0
12	26.6	32.5	32.3
13	13.1	17.4	17.5
1′	102.6	104.0	102.8
2′	74.8	75.2	75.0
3′	78.1	78.3	78.3
4′	71.6	71.7	71.9
5′	78.1	78.0	78.0
6′	62.8	62.8	63.0

Lasianthionosides B (**2**) was obtained as an amorphous powder, $[\alpha]_D$ −54.0°(MeOH) and C (3) as colorless needles, mp180–181 °C, $[\alpha]_D$ −55.7°(MeOH). Based on their negative- ion FAB mass spectra, both compounds have the same molecular formula, $C_{19}H_{32}O_8$, which is one oxygen atom less than that of lasianthionoside A (**1**). The ^{13}C NMR spectra of **2** and **3** were very similar and showed the absence of a quaternary carbon atom having an oxygen atom which was observed in **1**. The ^1H–^1H COSY spectra (in CD_3OD for **2** and C_5D_5N for **3**) of **2** (**3**) clearly demonstrated the connectivities from one of the dimethyl groups (δ1.06) (δ 1.37) to δ 1.74 (δ2.24)(2_{ax}-H)→δ 3.88 (δ 4.70) (H-3)→δ 3.72 (δ 4.33)(H-4)→δ 2.19 (δ 2.56) (H-5) which is coupled with the signals at δ 0.86 (δ 1.17) (H_3-13) and δ 2.10 (δ 2.37)(H-6). The connectivities from the latter signal (H-6) to δ 6.37 (δ 6.81)(H-7) and then to δ 6.06 (δ 6.11) (H-8) were also demonstrated. Cross peaks between 2_{eq}-H [δ 1.65 (δ 1.76)] and H-4, and H-6 and H_3-11 were further observed. The results together with consideration of the NOE experiments (Fig. 2), suggested that lasianthionosides B (**2**) and C (**3**) were 6-deoxy

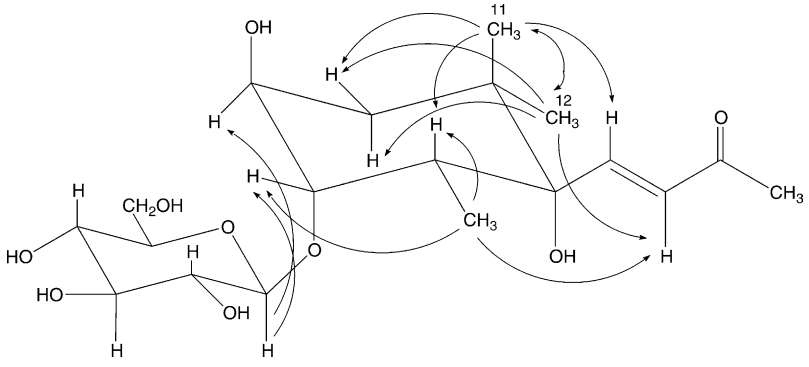

Fig. 1. The results of differential NOE experiments for lasianthionoside A (**1**).

derivatives and positional isomers in the glucopyranosyl moiety of lasianthionoside A (**1**). The locations of the glucosidic linkages were elucidated to be at *O*-3 in **2** and *O*-4 in **3** by comparison of ^{13}C NMR specral data with those of **1**. The stereochemistry of the glucosidic linkage was also elucidated as β as judged from the coupling pattern of the anomeric protons [δ 4.31 (*d*, $J=7.8$ Hz) in 2 and δ 4.93 (*d*, $J=7.8$ Hz) in 3]. Thus, the relative stereostructure of lasianthionosides B and C were elucidated as shown in (**2** and **3**).

To determine the absolute stereostructure, X-ray crystallographic analysis of lasianthionoside C (**3**) was performed. The result is shown in Fig. 3. Since enzymatic hydrolysis of **3** with β-D-glucosidase from almond gave D-glucose, the absolute stereostructure of lasianthionoside C has been determined as (3*S*, 4*S*, 6*S*, 7*E*)-3,4-dihydroxymegastigman-7-en-9-one-4-*O*-β-D-glucopyranoside. Considering the optical rotations and the very similar data of the ^{13}C NMR, lasianthionosides A (**1**) and B (**2**) may have the same absolute stereochemistry as that of lasianthionoside C (**3**).

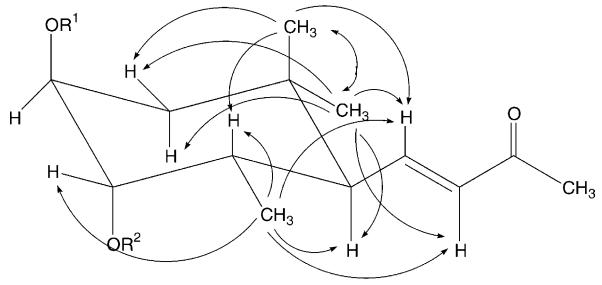

Fig. 2. The results of differential NOE experiments for lasianthionosides B (**2**) ($R^1=$Glc, $R^2=$H) and C (**3**) ($R^1=$H, $R^2=$Glc).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-360 polarimeter. FT-IR and UV spectra were recorded on Horiba FT-710 and JASCO V-530SR spectrophotometers, respectively. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Jeol JNM EX-400 spectrometer, using tetramethylsilane as internal standard. FABMS were obtained on a Jeol JMS SX-102 spectrometer using PEG-400 as calibration matrix. For purification, the following were used; Diaion HP-20

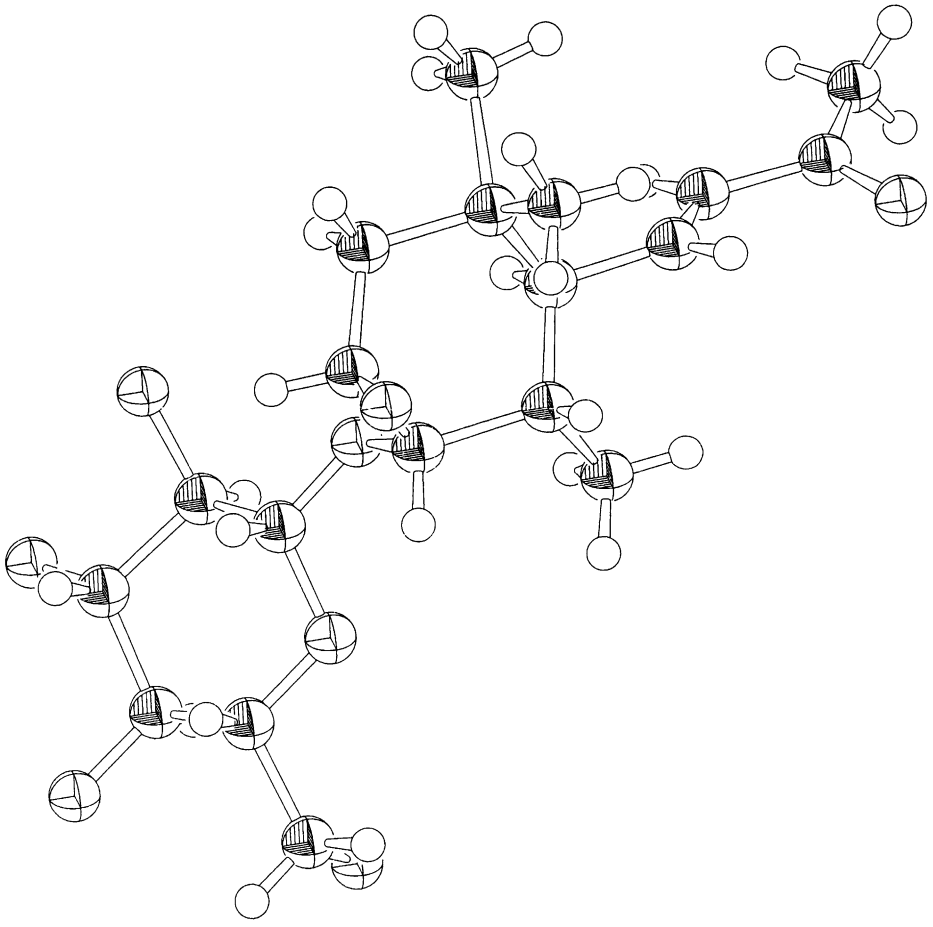

Fig. 3. Ortep plot of the crystal structure of lasianthionoside C (**3**).

(Mitsubishi Kagaku Co. Ltd., Tokyo), kiesel gel 60 Si gel (230–400 mesh, Merck), precoated silica gel plates 60 F_{254} (0.25 mm in thickness), packed column for HPLC (Cosmosil 10 C_{18} (20×250 mm), detection, 230 nm, solvent: MeOH–H_2O, 6 ml min^{-1}).

3.2. Plant material

Plant material was collected in July 1997, in Kunigamison, Okinawa Prefecture, Japan. A specimen was identified by one (T. S.) of the authors and a voucher herbarium specimen (97-LF-OKINAWA0710) is deposited in the Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University.

3.3. Isolation

Dried leaves (2.7 kg) of *L. fordii* were extracted with MeOH (2×80 l) for 2×2 weeks at room temp. The combined methanolic extract was concentrated in vacuo. The residue was dissolved in 90% aq MeOH and the solution was washed with *n*-hexane (1 l×3). The aq. MeOH layer was concentrated in vacuo. The residue was suspended in H_2O (1 l) and the suspension was extracted with EtOAc (1 l×3) and *n*-BuOH (1 l×3), successively. The *n*-BuOH layer was concentrated in vacuo to give a residue (15.7 g) which was applied to highly porous synthetic resin Diaion HP-20 (ϕ7.2×50 cm L) with a mixture of H_2O and MeOH with increasing MeOH content as eluant. Four liters each of 0, 30, 50 and 70% aq. MeOH and MeOH were eluted successively with 500 ml fraction, collected.

Fractions 13–16 were combined and concentrated, in vacuo to give a residue (0.759 g) which was purified by repeated silica gel chromatography (solvent: $CHCl_3$–MeOH) and finally HPLC (MeOH–H_2O 1:9) to give deacetylasperuloside (30.5 mg) and methyl deacetylasperulosidate (4.4 mg), respectively.

Fractions 27–31 were combined and concentrated in vacuo to give a residue (1.26 g) which was subjected to silica gel CC (60 g) with increasing amounts of MeOH in $CHCl_3$. Each 400 ml of 3, 5, 7, 10, 12, 15, 20 and 25% MeOH in $CHCl_3$ were eluted successively. Fractions of 50 ml were collected. Fractions 35–36 were combined and concentrated in vacuo to give asperuloside (646 mg). Fractions 45–51 were combined and evaporated in vacuo to give a residue (164 mg) which was separated by HPLC (solvent: MeOH–H_2O, 3:7) to give lasianthionosides A (1) (24.9 mg), B (2) (11.0 mg), C (3) (4.3 mg) and citroside A (9.7 mg), respectively.

The known iridoid glucosides, asperuloside, deacetylasperuloside and methyl deacetylasperulosidate were identified by direct comparisons of ^1H and ^{13}C NMR spectra with those of authentic samples and citroside A was identified by comparison of spectral data with those reported.

3.4. Lasianthionoside A (1)

Colorless needles, mp 209–210 °C. $[\alpha]_D^{25}$ −76.1°(C_5H_5N, *c* 0.87). UV λ_{max} (MeOH) nm (log ε): 232 (3.97), IR ν_{max} (film) cm^{-1}: 3340, 1666, 1641, 1365, 1269, 1078, 1047. ^1H NMR (C_5D_5N): δ 1.03 (3H, *s*, 1_{eq}-CH_3), 1.26 (3H, *d*, *J* = 6.8 Hz, H_3-13), 1.54 (3H, s, 1_{ax}-CH_3), 1.83 (1H, *br.d*, *J* = 16.1 Hz, 2_{eq}-H), 2.28 (3H, *s*, H_3-10), 2.57 (1H, *br.d*, *J* = 16.1 Hz, 2_{ax}-H), 2.90 (1H, *dq*, *J* = 2.4 and 6.8 Hz, H-5), 3.86 (1H, *m*, H-5′), 3.93 (1H, *dd*, *J* = 7.8 and 8.6 Hz, H-2′), 4.17 (1H, *dd*, *J* = 8.6 and 8.6 Hz, H-3′), 4.21 (1H, *dd*, *J* = 8.6 and 8.6Hz, H-4′), 4.36 (1H, *dd*, *J* = 11.7 and 5.4 Hz, Ha-6′), 4.49 (1H, *br.s*, H-4), 4.79 (1H, *br.s*, H-3), 4.94 (1H, *d*, *J* = 7.8 Hz, H-1′), 6.62 (1H, *br.s*, 3-OH), 6.82 (1H, *d*, *J* = 15.6 Hz, H-8), 7.09 (1H, *d*, *J* = 15.6 Hz, H-7); (CD_3OD): δ0.85 (3H. *s*. 1_{eq}-CH_3), 1.00 (3H, *d*, *J* = 7.3 Hz, H_3-13), 1.19 (3H, *s*, 1_{ax}-CH_3), 1.39 (1H, *br.d*, *J* = 14.9 Hz, 2_{eq}-H), 2.07 (1H, *dd*, *J* = 14.9 and 2.7 Hz, 2_{ax}-H), 2.28 (3H, s, H_3-10), 2.49 (1H, *dq*, *J* = 2.9 and 7.3 Hz, H-5), 3.14 (1H, *dd*, *J* = 8.8 and 7.8 Hz, H-2′), 3.21-3.38 (3H, H-3′,4′,5′), 3.66 (1H, *dd*, *J* = 11.7 and 5.4 Hz, Ha-6′), 3.86 (1H, *dd*, *J* = 11.7 and 2.0 Hz, Hb-6′),3.86 (1H, *br.s*, H-4), 4.08 (1H, *br.d*, *J* = 2.9 Hz, H-3), 4.28 (1H, *d*, *J* = 7.8 Hz, H-1′), 6.32 (1H, *d*, *J* = 15.6 Hz, H-8) and 6.86 (1H, *d*, *J* = 15.6 Hz, H-7). For ^{13}C NMR see Table 1. HR-FAB-MS (negative): *m*/*z* 403.1956 ($C_{19}H_{31}O_9$ requires 403.1968).

3.5. Lasianthionoside B (2)

Amorphous powder, $[\alpha]_D^{25}$ −54.0° (MeOH, *c* 0.55). UV λ_{max} (MeOH) nm (log ε): 231.5 (4.04). IR ν_{max} (film) cm^{-1} : 3371, 1660, 1622, 1365, 1261, 1167, 1076, 1039. ^1H NMR (C_5D_5N):δ 0.84 (3H, *s*, 1_{eq}-Me), 1.09 (3H, *d*, *J* = 6.4 Hz, H_3-13), 1.24 (3H, *s*, 1_{ax}-Me), 1.84 (1H, *br.d*, *J* = 14.2 Hz, 2_{eq}-H), 2.14 (1H, *dd*, *J* = 14.2 and 3.4 Hz, 2_{ax}-H), 2.50 (2H, H-5,6), 3.89 (1H, *m*, H-5′), 4.07 (1H, *dd*, *J* = 7.8 and 8.3 Hz, H-2′), 4.22 (1H, *dd*,*J* = 8.3 and 8.3 Hz, H-3′), 4.27 (1H, *dd*, *J* = 8.3 and 8.3 Hz, H-4′), 4.33 (1H, *br.s*, H-4), 4.02 (1H, *dd*, *J* = 11.7 and 5.4 Hz, H_a-6′), 4.54 (1H, *br.s*, H-3), 4.55 (1H, *br.d*, *J* = 11.7 Hz, H_b-6′), 4.99 (1H, *d*, *J* = 7.8 Hz, H-1′), 6.23 (1H, *d*, *J* = 15.6 Hz, H-8), 6.41 (1H, *br.s*, OH), 6.87 (1H, *dd*, J = 15.6 and 10.0 Hz, H-7); (CD_3OD): δ0.78 (3H, *s*, 1_{eq}-CH_3), 0.86 (3H, *d*, *J* = 6.8 Hz, H_3-13), 1.06 (3H, *s*, 1_{ax}-CH_3), 1.65 (1H, *dd*, *J* = 14.7 and 2.4 Hz, 2_{eq}-H), 1.74 (1H, *dd*, *J* = 14.7 and 3.7 Hz, 2_{ax}-H), 2.10 (1H, *dd*, *J* = 10.7 and 10.7 Hz, H-6), 2.19 (1H, *m*, H-5), 2.26 (3H, *s*, H_3-10), 3.16 (1H, *dd*, *J* = 8.3 and 7.8 Hz, H-2′), 3.22-3.40 (3H, H-3′,4′,5′),3.67 (1H, *dd*, *J* = 11.7 and 5.4 Hz, H_a-6′), 3.72 (1H, *br.s*, H-4), 3.88 (1H, *br.s*, H-3), 3.85-3.88 (1H, overlapped, H_b-6′), 4.31 (1H, *d*, *J* = 7.8 Hz, H-1′), 6.06 (1H, *d*, *J* = 15.9 Hz, H-8), 6.73 (1H, *dd*, *J* = 15.9 and 10.7 Hz, H-7). For ^{13}C NMR see Table 1. HR-FABMS (negative): *m*/*z* 387.2031 ($C_{19}H_{31}O_8$ requires 387.2019).

3.6. Lasianthionoside C (3)

Colorless needles, mp 180–181 °C, $[\alpha]_D^{25}$ −55.7 °C(MeOH, *c* 0.26). UV λ_{max} (MeOH)nm (log ε): 231(4.04). IR ν_{max} (film) cm^{-1}: 3340, 1657, 1621, 1423, 1365, 1265, 1165, 1076, 1036. ^1H NMR (C_5D_5N):δ 0.72 (3H, *s*, 1_{eq}-CH_3), 1.17 (3H, *d*, *J* = 6.8 Hz, H_3-13), 1.37 (3H, *s*, 1_{ax}-CH_3), 1.76 (1H, *br.d*, *J* = 14.2 Hz, 2_{eq}-H), 2.24 (1H, *br.d*, *J* = 14.2 Hz, 2_{ax}-H), 2.25 (3H, *s*, H_3-10), 2.37 (1H, *dd*, *J* = 10.7 and 10.7 Hz, H-6), 2.56 (1H, *m*, H-5), 3.86 (1H, *m*, H-5′), 4.06 (1H, *dd*, *J* = 8.3 and 7.8 Hz, H-2′), 4.20 (1H, *dd*, *J* = 8.3 and 8.3 Hz, H-3′), 4.25 (1H, *dd*, *J* = 8.3 and 8.3 Hz, H-4′), 4.33 (1H, *br.s*, H-4), 4.38 (1H, *dd*, *J* = 11.7 and 5.4 Hz, H_a-6′), 4.54 (1H, *dd*, *J* = 11.7 and 2.4 Hz, H_b-6′), 4.70 (1H, *br.d*, *J* = 2.9 Hz, H-3), 4.93 (1H, *d*, *J* = 7.8 Hz, H-1′), 6.11 (1H, *d*, *J* = 15.6 Hz, H-8), 6.47 (1H, *br.s*., OH), 6.81 (1H, *dd*, *J* = 15.6 and 10.7 Hz, H-7); (CD_3OD):δ0.82 (3H, *s*, 1_{eq}-Me), 0.90 (3H, *d*, *J* = 6.3 Hz, H_3-13), 1.46 (1H, *br.d*, *J* = 14.6 Hz, 2_{eq}-H), 1.86 (1H, *dd*, *J* = 14.6 and 3.2 Hz, 2_{ax}-H), 2.17 (2H, H-5, H-6), 2.26 (3H, *s*, H_3-10), 3.15 (1H, *dd*, *J* = 8.3 and 7.8 Hz, H-2′), 3.20-3.35 (3H, H-3′,4′,5′), 3.65 (1H, *dd*, *J* = 12.0 and 5.6 Hz, H_a-6′), 3.70 (1H, *br.s*, H-4), 3.85 (1H, *dd*, *J* = 12.2 and 2.2 Hz, H_b-6′), 4.01 (1H, *br.d*, *J* = 3.2 Hz, H-3), 4.27 (1H, *d*, *J* = 7.8 Hz, H-1′), 6.03 (1H, *d*, *J* = 15.6 Hz, H-8), 6.72 (1H, *dd*, *J* = 15.6 and 10.3 Hz, H-7). For ^{13}C NMR, see Table 1. HR-FAB-MS (negative): *m*/*z* 387.1998 ($C_{19}H_{31}O_8$ requires 387.2019).

3.7. Enzymatic hydrolysis of lasianthionoside C (3)

2.5 mg of lasianthionoside C (**3**) was dissolved in H_2O (1 ml) containing DMSO (0.1 ml) and β-D-glucosidase (10 mg) from almond was added to the solution. The mixture was incubated for 24 h at 37 °C and washed with EtOAc. The aqueous layer was concentrated in vacuo, to give a residue which showed a spot of glucose (R_f 0.32: *n*-BuOH–Me_2CO–H_2O 4:5:1) on silica gel TLC. The residue was converted into a thiazolidine derivative and analysed by silica gel TLC (R_f 0.49 and 0.38, $CHCl_3$–MeOH–H_2O 15:6:1) (Miyaichi and Tomimori, 1998). Authentic thiazolidine derivatives obtained from D- and L-glucoses showed spots at R_f 0.49 and 0.38, and 0.45, respectively.

3.8. X-ray analysis of lasianthionoside C (3)

Crystal data: $C_{19}H_{32}O_8{\cdot}2H_2O$, M = 424.49, triclinic, space group *C* 1, *a* = 23.48(1) Å, *b* = 6.078(3) Å, *c* = 19.181(10) Å, α = 89.68(5)°, β = 121.88(3)°, γ = 92.19(5)°, *V* = 2323(2) $Å^3$, *Z* = 4, *D*c = 1.214 Mgm^{-3}, *F*(000) = 920, μ(Mo*K*α) = 0.973 cm^{-3}.

The crystal used for data collection was a colourless needle with approximate dimensions of 0.5×0.2×0.1 mm. All data were obtained on a Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo*K*α radiation. Unit cell parameters were determined by least squares refinement of the optimized setting angles of 25 reflections in the range of $7.5° < \theta < 9.5°$. The intensities were measured using $\omega/2\theta$ scan up to 55°. Three standard reflections were monitored every 150 measurements. The data were corrected for Lorentz and polarization factors. A correction for secondary extinction was applied (coefficient = 0.31242×10^{-7}). Decay (−10.85% decline) correction was applied. Of the 5832 reflections which were collected, 5329 unique reflections were used for structure determination and refinement. The structure was solved by a direct method using teXsan crystallographic software package (teXsan, 2000). All non-H atoms were found in a Fourier map. The refinement of atomic parameters were carried out by the fullmatrix least-squares refinement, using anisotropic temperature factors for all non-H atoms. All H atoms, except those attached to O atoms, were located geometrically and not refined. The H atom attached to O atoms were not found in the Difference Fourier map. The final refinement converged with $R_1 = 0.055$ and $Rw = 0.186$ for 518 parameters. Atomic scattering factors were taken from "International Tables for X-ray Crystallography." Crystallographic data for lasianthiomoside C (**3**) have been deposited in the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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