# An Iridoid Glucoside Dimer and a Non-glycosidic Iridoid from the Leaves of *Lasianthus wallichii*

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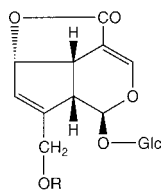
**A new iridoid glucoside dimer (1) and a non-glycosidic iridoid (2) was isolated together with the known compounds, asperuloside (3), paederoside (4), daphylloside (5), citroside A (6) and benzyl 6-*O*-$\alpha$-L-rhamnopyranosyl-$\beta$-D-glucopyranoside (7), from the leaves of *Lasianthus wallichii*. The structures of the new compounds were elucidated by spectroscopic and chemical evidence.**

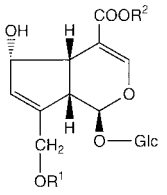
**Key words** *Lasianthus wallichii*; Rubiaceae; iridoid glucoside dimer; non-glycosidic iridoid

Genus *Lasianthus* is classified in the tribe Morindeae, subfamily Rubioideae (Rubiaceae). Kooiman[1] pointed out the presence of so called asperulosidic acid in almost all Rubioideae plants except for some exceptions based on the test of the extracts with the Trim and Hill reaction.[2] Although the presence of asperuloside (**3**) in some of plants of the genus *Lasianthus* was reported[3] based on the results of TLC and GC of the glycosidic fraction, few reports dealing with the constituents have appeared. In the course of studies on the constituents of the plants which are grown under a subtropical climate, we investigated the constituents of the leaves of *Lasianthus wallichii* (WIGHT & ARN.) Wight (Rubiaceae) and isolated two new compounds, one of which is bis-iridoid glucoside (**1**) and the other non-glycosidic iridoid (**2**), together with five known compounds: asperuloside (**3**),[4] paederoside (**4**),[5,6] daphylloside (**5**),[7] citroside A (**6**),[8] benzyl 6-*O*-$\alpha$-L-rhamnopyranosyl-$\beta$-D-glucopyranoside (**7**).[9] This paper deals with the isolation and structure elucidation of the new compounds.

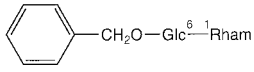
Compound 1 (**1**) was isolated as an amorphous powder, $[\alpha]_D$ −58.4° (MeOH) and the molecular formula was assigned as $C_{36}H_{44}O_{22}$, based on its negative ion high resolution (HR)-FAB-MS. It showed an absorption maximum at 228 nm ($\varepsilon$ 14120) in the UV spectrum. The $^1$H-NMR spectrum showed, in addition to two singlets at $\delta$ 2.05 and 2.08 (each 3H), two signals at $\delta$ 7.30 (1H, d, $J$=2.2 Hz) and 7.70 (1H, dd, $J$=1.7, 0.6 Hz), which are characteristic of the proton on C-3 of iridoid glucosides having a carbonyl group on C-4, two signals at $\delta$ 5.07 (1H, dd, $J$=9.0, 0.6 Hz) and 5.83 (1H, dd, $J$=0.6, 0.6 Hz) assigned to acetalic protons and two anomeric protons at $\delta$ 4.73 (2H, d, $J$=7.9 Hz). Thus compound 1 was presumed to be composed of two iridoid glycoside units. The structure of the aglycone portion of one unit was shown to be the same as that of **3** (partial structure A) on the results of $^1$H–$^1$H correlation spectroscopy (COSY) spectrum. Thus, starting from the signal at $\delta$ 7.30 assignable to the proton on C-3, cross peaks were followed to $\delta$ 3.67 (H-5), 5.56 (H-6), 5.73 (H-7), and 4.63 and 4.72 ($H_2$-10). In contrast, starting from the signal at $\delta$ 3.67, cross peaks were followed to $\delta$ 3.23 (H-9) and 5.83 (H-1), successively. The structure of the aglycone of the other unit was also elucidated to be the same as that of 10-*O*-acetylasperulosidate (partial structure B) based on the results of $^1$H–$^1$H COSY spectrum. Thus, starting from the signal at $\delta$ 7.70, cross peaks were followed to the signals at $\delta$ 3.05 (H-5), 4.84 (H-6), 6.02 (H-7), and 4.80 and 4.94 ($H_2$-10), successively. The cross peaks were further followed to the signals at $\delta$ 2.66 (H-9) and 5.07 (H-1) starting from the signal at $\delta$ 3.05. The $^{13}$C-NMR data shown in Table 1 clearly demonstrated that compound 1 is constituted from **3** unit and 10-*O*-acetylasperulosidate unit. Since the C-11 signal of unit B resonated in almost the same region as in **5** (Table 1), the carboxyl group at C-4 in the partial structure unit B formed an ester linkage with one of the hydroxyl group in the glucose moiety in the partial structure of unit A. Alkaline hydrolysis of compound 1 gave deacetylasperulosidic acid (**8**).[11] The location of the ester linkage was inferred at *O*-6′ in unit A, because the signal assigned to $H_2$-6′ of that unit suffered downfield shift to $\delta$ 4.22 and 4.63 and $^{13}$C-NMR signals of sugar portion for unit A well correspond to those of 6-*O*-acylated glucose derivatives.[10] This




(**3**) R=COMe
(**4**) R=COS·Me




(**5**) $R^1$=COMe ; $R^2$=$CH_3$
(**8**) $R^1$=$R^2$=H




(**7**)

Glc : β-D-glucopyranosyl
Rham : α-L-rhamnopyranosyl

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Table 1. $^{13}$C-NMR Data ($\delta$, ppm)[a] of Compound 1 (**1**), Asperuloside (**3**) and Daphylloside (**5**) (in $CD_3OD$)

| Carbon | **1** | | **3** | **5** |
|---|---|---|---|---|
| | Unit A | Unit B | | |
| 1 | 93.4 | 101.5 | 93.3 | 101.3 |
| 3 | 150.2 | 155.8 | 150.3 | 155.4 |
| 4 | 106.4 | 108.2 | 106.2 | 108.1 |
| 5 | 37.5 | 42.6 | 37.5 | 42.4 |
| 6 | 86.3 | 75.6 | 86.3 | 75.4 |
| 7 | 129.3 | 132.0 | 128.9 | 131.8 |
| 8 | 144.2 | 146.1 | 144.2 | 146.0 |
| 9 | 45.4 | 46.4 | 45.2 | 46.2 |
| 10 | 61.9 | 63.7 | 60.9 | 63.8 |
| 11 | 172.1 | 168.7 | 172.2 | 169.3 |
| Ac | 20.8 | 20.8 | 20.7 | 20.8 |
| | 172.5 | 172.5 | 172.5 | 172.5 |
| 1′ | 100.1 | 100.7 | 100.0 | 100.6 |
| 2′ | 74.7 | 75.0 | 74.6 | 74.9 |
| 3′ | 78.0 | 78.6 | 78.3 | 78.6 |
| 4′ | 71.7[b] | 71.8[b] | 71.5 | 71.5 |
| 5′ | 76.0 | 77.9 | 77.8 | 77.9 |
| 6′ | 64.4 | 63.1 | 62.8 | 63.0 |

*a*) The assignments are based on $^1$H-COSY, HSQC, HMBC and comparisons of spectra of closely related compounds. *b*) May be interchanged.

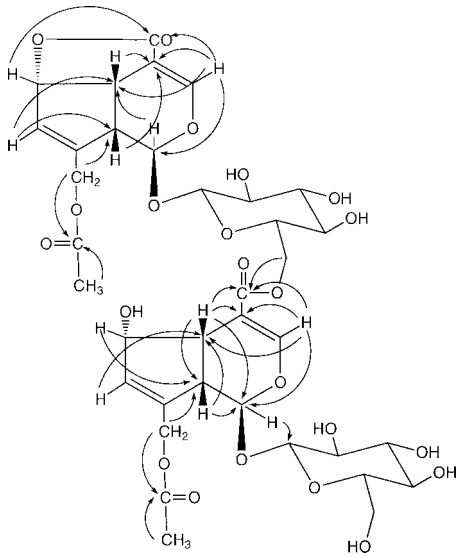

Fig. 1. The Results of HMBC Spectrum of Compound 1 (**1**) ($J$=8 Hz)

presumption was confirmed by the facts that the proton signals of $H_2$-6′ of unit A crossed peaks with the ester carbonyl signal at $\delta$ 168.7 (C-11 in unit B) which crossed peaks with the signal due to H-3$_B$ and H-5$_B$ in the unit B in the heteroatom multiple bond correlation (HMBC) spectrum, the results of which are shown in Fig. 1. Thus, the structure of compound 1 was elucidated as shown in Fig. 1.

Compound 2 (**2**) was isolated as a syrup, $[\alpha]_D$ −30.6° (MeOH). The molecular formula was assigned as $C_{10}H_{14}O_5$ based on its negative ion HR-FAB-MS. The compound was transparent above 220 nm in the UV spectrum and showed absorptions of hydroxyl groups (3332 cm$^{-1}$) and a five-membered lactone group (1747 cm$^{-1}$) in the IR spectrum. The $^{13}$C-NMR spectrum (Table 2) showed only ten carbon signals composed of four methylene groups, three of which bear a

Table 2. $^1$H- and $^{13}$C-NMR Data ($\delta$) of Compound 2 (**2**) (in $CD_3OD$)

| Atom | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 58.5 | 4.19 (2H, br s) |
| 3 | 63.5 | 3.86 (dd, $J$=10.6, 3.7 Hz) |
| | | 3.93 (dd, $J$=10.6, 4.1 Hz) |
| 4 | 48.7 | 2.83 (ddd, $J$=6.4, 4.1, 3.7 Hz) |
| 5 | 53.2 | 3.67 (m) |
| 6 | 83.2 | 5.11 (td, $J$=6.6, 1.1 Hz) |
| 7 | 41.9 | 2.71 (d, $J$=18.1 Hz) |
| | | 2.91 (ddd, $J$=18.1, 6.6, 1.1 Hz) |
| 8 | 138.5 | |
| 9 | 137.4 | |
| 10 | 57.1 | 4.15 (br d, $J$=13.2 Hz) |
| | | 4.29 (br d, $J$=13.2 Hz) |
| 11 | 180.8 | |

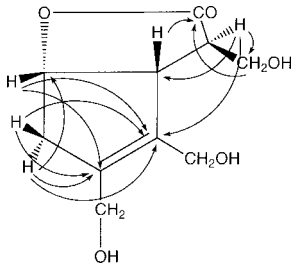

Fig. 2. The Results of HMBC Spectrum of Compound 2 (**2**) ($J$=8 Hz)

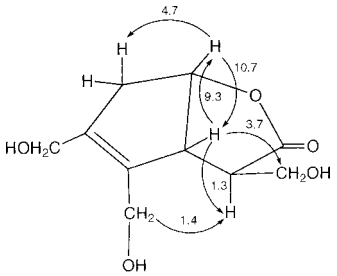

Fig. 3. The Results of NOE Experiments for Compound 2 (**2**)

hydroxyl group; three methine groups, one of which bears an carbonyloxy group; a tetrasubstituted double bond and a lactonic carbon atom. Analysis of one-dimensional spectra and $^1$H-COSY, heteronuclear single quantum coherence (HSQC) and HMBC (Fig. 2) spectra led to the proposed structure for compound 2 and enabled the complete assignments of $^1$H- and $^{13}$C-NMR signals. The observed coupling constant in the $^1$H-NMR spectra and the co-occurrence of **3** which might be a possible biogenetic precursor strongly suggested that chiral centers at C-5 and 6 are *R* and *S*-configurations. The results of differential nuclear Overhauser effect (NOE) experiments shown in Fig. 3 and CD spectrum support the above presumption and the stereochemistry at C-4 is *R*-configuration.[12] Thus, the structure of compound 2 was elucidated as shown in Fig. 2.

**Experimental**

Optical rotations were measured on a JASCO DIP-360 digital polarimeter. IR spectra were measured on a Perkin-Elmer IR spectrometer and UV spectra on a JASCO V-530 SR spectrophotometer. $^1$H- and $^{13}$C-NMR spectra were taken on a JEOL JNM EX-400 or $\alpha$-400 spectrometer at 400 and 100 MHz, respectively, with tetramethylsilane as an internal standard. HR-

FAB-MS were performed on a JEOL JMS SX-102 spectrometer with polyethylene glycol-400 as a matrix. Column chromatography was performed on Diaion HP-20 (Mitsubishi Kagaku Co., Ltd., Tokyo), silica gel 60 (230—400 mesh, Merck), and octadecyl silica (ODS) gel (Cosmosil $75C_{18}$-OPN, Nacalai Tesque, Kyoto), and TLC was performed on precoated silica gel plates 60 $F_{254}$ (0.25 mm in thickness, Merck). HPLC was performed on ODS gel (Cosmosil $10C_{18}$, Nacalai Tesque, Kyoto, $\phi$=20 mm, L=250 mm) with a mixture of $H_2O$ and MeOH at the flow rate of 6 ml $min^{-1}$ and the eluate was monitored by UV.

**Plant Material** Plant material was collected in Kunigami-son, Kunigami-gun and Taketomi-cho, Yaeyama-gun, Okinawa Prefecture in July, 1998 and in October, 2000, respectively, and identified as *Lasianthus wallichii* (Wight & Arn.) Wight by one (T.S.) of the authors . Voucher specimens (98-LW-Okinawa-0709 and 00-LW-Okinawa-1004) were deposited in the Herbarium of the Department of Pharmacognosy, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University. In this report, we used the sample collected in Kunigami-gun, since the TLC pattern of *n*-BuOH soluble fractions obtained from both samples is the same.

**Extraction and Isolation** Dried leaves (3.9 kg) of *L. wallichii* were extracted with MeOH (72 l) at room temperature for 2 weeks. Extraction was repeated once in the same manner. The combined methanolic extract was concentrated *in vacuo*. The residue was dissolved in 90% MeOH (1.1 l) and the solution was washed with *n*-hexane (1 l×3). The 90% MeOH layer was concentrated *in vacuo*. The residue was suspended in $H_2O$ and the suspension was extracted with EtOAc (1 l×3). The aqueous layer was extracted with *n*-BuOH (1 l×3). The *n*-BuOH extract was evaporated *in vacuo* to give a residue (70.5 g).

The residue was chromatographed on Diaion HP-20 (70 mm in diameter and 445 mm in length). Adsorbed material was eluted successively with $H_2O$–MeOH with a stepwise increase of MeOH content. Three and one-half liters of 0%, 20%, 40% and 60% MeOH in $H_2O$ and MeOH were eluted successively, and 500 ml fractions were collected. The residue (2.62 g) of fractions 11—14 was subjected to silica gel (55 g) column chromatography. Five hundred milliliters of each of $CHCl_3$ and $CHCl_3$–MeOH (97 : 3, 19 : 1, 9 : 1, 22 : 3, 17 : 3, 4 : 1, 3 : 1, 7 : 3) were eluted successively. The eluate (520 mg) from 5—10% MeOH–$CHCl_3$ which contained a spot (*Rf* 0.30, solvent: $CHCl_3$–MeOH–$H_2O$ 15 : 6 : 1) on TLC was separated by HPLC (solvent: MeOH–$H_2O$ 1 : 9, detection 210 nm) to give compound 2 (94.7 mg) as a viscous gum.

The residue (10.1 g) of fractions 15—24 of Diaion HP-20 column was chromatographed over silica gel (500 g). Three liters of $CHCl_3$ and $CHCl_3$–MeOH (97 : 3, 19 : 1, 93 : 7, 9 : 1, 22 : 3, 17 : 3, 4 : 1, 3 : 1, 7 : 3) were eluted successively. From the eluate of $CHCl_3$–MeOH (9 : 1), 300 ml fractions were collected. An aliquot (300 mg) of the residue (863 mg) from fractions 11—16 was separated by HPLC (solvent: MeOH–$H_2O$ 3 : 7; detection 210 nm) to give asperuloside (**3**) (23.2 mg) and citroside A (**6**) (14.4 mg). Fractions 17—21 gave a residue (742 mg), an aliquot (152 mg) of which was separated by HPLC (solvent: MeOH–$H_2O$ 7 : 13; detection 210 nm) to give benzyl alcohol 6′-$\alpha$-L-rhamnopyranosyl-$\beta$-D-glucopyranoside (**7**) (19.6 mg). An aliquot (199 mg) of the residue (578 mg) was separated by HPLC (solvent: MeOH–$H_2O$ 3 : 7; detection 210 nm) to give another aliquot of asperuloside (**3**) (18.6 mg) and benzyl 6-*O*-$\alpha$-L-rhamnopyranosyl-$\beta$-D-glucopyranoside (**7**) (23.3 mg).

Fractions 25—32 of Diaion HP-20 column chromatography gave a residue (33.2 g) which was chromatographed over silica gel (1 kg) with $CHCl_3$–MeOH as eluent with increasing amounts of MeOH content $CHCl_3$ (4.5 l), $CHCl_3$–MeOH (9 : 1, 4.5 l), $CHCl_3$–MeOH (22 : 3, 4.5 l), $CHCl_3$–MeOH (17 : 3, 6.5 l), and $CHCl_3$–MeOH (4 : 1, 5.5 l) were eluted successively, 500 ml fractions were collected. Fractions 19—22 gave a residue (698 mg), an aliquot (110 mg) of which was separated by HPLC (solvent: MeOH–$H_2O$ 7 : 13, detection 230 nm) to give paederoside (**4**) (19.7 mg) and daphylloside (**5**) (6.3 mg). Fractions 23—25 gave a residue (615 mg). An aliquot (100 mg) was separated by HPLC (solvent: MeOH–$H_2O$ 7 : 13, detection 230 nm) to give **3** (12.4 mg), **4** (11.1 mg) and **5** (8.1 mg). Fractions 35—40 gave a residue (4.20 g) which was chromatographed over ODS gel (45 mm in diameter and 470 mm in length). Adsorbed material was eluted with $H_2O$–MeOH with a stepwise increase of 50% MeOH content (4.5 l) and 60% MeOH (4.5 l) eluted successively, collecting 100 ml fractions. Fractions 6—7 gave a residue (1.07 g) which was further separated by silica gel (55 g) column chromatography with $CHCl_3$–MeOH as eluent with an increasing amount of MeOH in $CHCl_3$ and finally by HPLC (solvent: MeOH–$H_2O$ 7 : 13, detection 230 nm) to give compound 1 (**1**) (36.3 mg).

The known compounds isolated, asperuloside (**3**), paederoside (**4**), daphylloside (**5**), citroside A (**6**) and benzyl 6-*O*-$\alpha$-L-rhamnopyranosyl-$\beta$-D-glucopyranoside (**7**) were identified with authentic samples by direct comparison or by comparison of their spectral data with those reported. The physical properties of the new compounds are as follows.

Compound 1 (**1**): an amorphous powder, $[\alpha]_D^{27}$ −58.4° (*c*=0.66, MeOH). UV $\lambda_{max}$ (MeOH) nm ($\varepsilon$): 228 (14120). IR $\nu_{max}$ (film) $cm^{-1}$: 3382, 1732, 1658, 1633, 1257, 1053, 1026. $^1$H-NMR ($CD_3OD$): 2.05, 2.08 (each 3H, s, 2×OAc), 2.66 (1H, ddd, *J*=8.2, 8.2, 0.6 Hz, H-$9_B$), 3.05 (1H, ddd, *J*=8.2, 6.0, 1.7 Hz, H-$5_B$), *ca.* 3.23 (H-$9_A$), 3.61 (1H, dd, *J*=11.7, 4.0 Hz, $H_1$-6′$_B$), 3.60—3.66 (2H, m, H-5′$_A$, H-5′$_B$), 3.67 (1H, ddd, *J*=6.5, 6.5, 2.2 Hz, H-$5_A$), 3.85 (1H, dd, *J*=11.9, 1.8 Hz, $H_1$-6′$_B$), 4.22 (1H, dd, 11.9, 6.2 Hz, $H_1$-6′$_A$), 4.63 (1H, dd, *J*=11.9, 2.2 Hz, $H_1$-6′$_A$), 4.63 (1H, dd, *J*=14.1, 1.2 Hz, $H_1$-$10_A$), 4.72 (1H, dd, *J*=14.1, 1.2 Hz, $H_1$-$10_A$), 4.73 (2H, d, *J*=7.9 Hz, H-1′$_A$ and H-1′$_B$), 4.80 (1H, dd, *J*=15.0, 1.8 Hz, $H_1$-$10_B$), 4.84 (1H, dd, *J*=6.0, 2.6 Hz, H-$6_B$), 4.94 (1H, dd, *J*=15.0, 1.8 Hz, $H_1$-$10_B$), 5.07 (1H, dd, *J*=9.0, 0.6 Hz, H-$1_B$), 5.56 (1H, dt, *J*=6.5, 1.7 Hz, H-$6_A$), 5.73 (1H, m, H-$7_A$), 5.83 (1H, d, *J*=0.6 Hz, H-$1_A$), 6.02 (1H, br d, *J*=1.8 Hz, H-$7_B$), 7.30 (1H, d, *J*=2.2 Hz, H-$3_A$), 7.70 (1H, dd, *J*=1.7, 0.6 Hz, H-$3_B$). $^{13}$C-NMR: see Table 1. HR-FAB-MS (negative) *m/z*: 827.2258 $[M-H]^-$ (Calcd for $C_{36}H_{43}O_{22}$: 827.2246).

Compound 2 (**2**): A viscous syrup, $[\alpha]_D^{28}$ −30.6° (*c*=0.72, MeOH). IR $\nu_{max}$ (film) $cm^{-1}$: 3332, 1747, 1362, 1192, 1061, 991. $^1$H- and $^{13}$C-NMR: see Table 2. CD $\lambda_{max}$ (MeOH) nm ($\Delta\varepsilon$): 286 (−0.013), 218 (+1.61). HR-FAB-MS (negative) *m/z*: 213.0783 $[M-H]^-$ (Calcd for $C_{10}H_{13}O_5$: 213.0763).

**Alkaline Hydrolysis of Compound 1 (1)** **1** (13.1 mg) was dissolved in 0.5 N NaOH aqueous solution (1 ml) and the solution was stirred for 4.5 h at room temperature. The solution was neutralized with an ion-exchange resin, Amberlite IR-120B (H-form). The ion exchange resin was filtered off and the filtrate was concentrated *in vacuo* to give **8** (10.5 mg).[11]

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