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Iridoid Glucosides from *Lamium amplexicaule*

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The iridoid glucosides lamioside (**1**), lamalbid (**8**), shanzhiside methyl ester (**9**) and barlerin (**10**) were isolated from the whole plant of *Lamium amplexicaule* L. The preferred conformations of the cyclopentane ring of the acetates (**1a**, **8a**, **9a** and **10a**) in solution were deduced from extensive proton nuclear magnetic resonance spectral analyses.

Keywords——*Lamium amplexicaule*; Labiatae; iridoid glucoside; lamioside; lamalbid; shanzhiside methyl ester; barlerin

In previous papers,[1-4] we reported the isolation and structural determination of iridoid glucosides and phenyl propanoid glycosides from *Campsis chinensis* (Bignoniaceae). As a continuation of our studies on the constituents of iridoid glycosides, we examined the constituents of iridoid glycosides of *Lamium amplexicaule* (Labiatae). There are reports of the isolation of various iridoid glucosides, lamioside (**1**),[5] lamiol (**2**),[5] lamiide (**3**),[6] ipolamiide (**4**),[6,7] ipolamiidoside (**5**),[7] 5-deoxylamioside (**6**)[8] and 6-deoxylamioside (**7**)[9] from this plant. Careful reinvestigation of a methanol extract of the whole plant of *L. amplexicaule* led to the isolation of lamalbid (**8**), shanzhiside methyl ester (**9**) and barlerin (**10**), together with **1**.

We describe here the preferred conformations of the cyclopentane ring in the acetates of these compounds in solution, as well as the identification of the four iridoid glycosides, **1** and **8**—**10**.

The four iridoid glucoside, L-sides I—IV, were isolated as described in Experimental.

L-side I (**1**), a white powder, $[\alpha]_D$ −58.4° (MeOH), gave D-glucose and a black product (derived from the aglycone) on acid hydrolysis, and gave a pentaacetate (**1a**), mp 204—206 °C, on acetylation with acetic anhydride and pyridine. L-side I was established to be lamioside (**1**),[5] by comparison of the physical and spectral data for **1** and **1a** with reported values.[5]

L-side II (**8**) was isolated as a white powder, $[\alpha]_D$ −101.5° (MeOH), and gave a hexaacetate (**8a**), mp 150—151 °C, on acetylation with acetic anhydride and pyridine. The infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectral data for **8** and **8a** were identical with those for lamalbid and its hexaacetate from *Lamium album* L. (Labiatae).[10]

L-side III (**9**) was isolated as a white powder, $[\alpha]_D$ −81.0° (MeOH), and gave a pentaacetate (**9a**), mp 177—179 °C, and a hexaacetate (**9b**), mp 184—187 °C, on acetylation with acetic anhydride and pyridine at 50 °C. The physical and spectral data of **9** and **9a** were identical with those of shanzhiside methyl ester and its pentaacetate from *Mussaenda parbiflora* (Rubiaceae).[11]

L-side IV (**10**) was obtained as a white powder, $[\alpha]_D$ −81.0° (MeOH), which gave D-glucose together with a black product due to decomposition of the aglycone on acid hydrolysis. The IR and ^{1}H-NMR spectral data showed the presence of a typical iridoid structure[12] possessing an α, β unsaturated methoxycarbonyl group [IR(KBr): 1710,

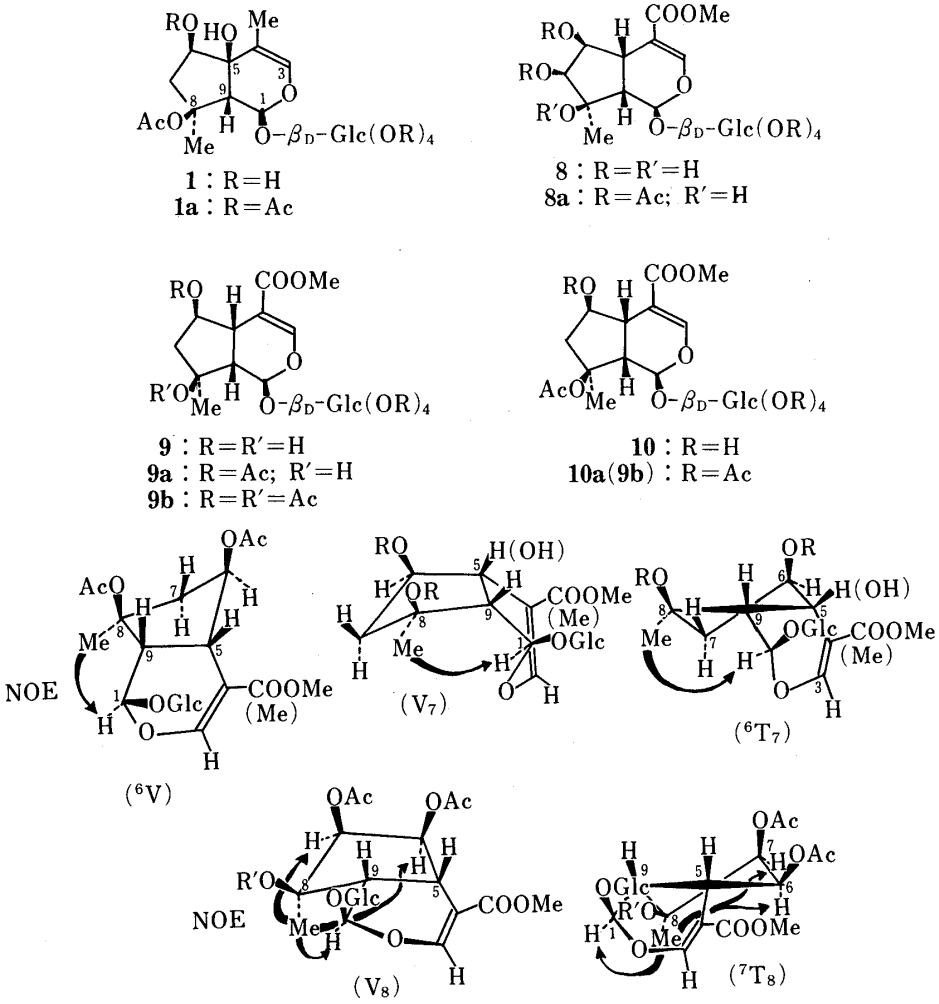

Chart 1

1630 cm^{-1}; ^{1}H-NMR (CD_3OD) δ: 3.68 (s)], a deshielded tertiary methyl group [δ 1.84 (s)], an acetyl group [1720 cm^{-1}; δ 1.96 (s)] and a β-D-glucopyranosyl moiety [3400 cm^{-1}; δ 4.57 (d, J=8.0 Hz)]. Acetylation of **10** with acetic anhydride and pyridine gave a pentaacetate (**10a**), mp 187—189 °C, $[\alpha]_D$ −147.4° ($CHCl_3$), which did not contain a hydroxyl group, judging from its IR spectrum. The above results and the ^{1}H-NMR spectral data (see Table II and Experimental) on **10a** indicated the presence of a β-hydroxyl group at C-6, and an acetoxyl and a tertiary methyl group at C-8 in **10**. The configurations of the acetoxyl and tertiary methyl groups at C-8 were concluded to be β and α, respectively, for the following reasons. As seen in other C-8β-acetoxyl substituted/C-8β-hydroxyl substituted pairs of iridoid glucosides,[4,13] the signals of H-1, H-9, and Me-8 in **10** were 0.25, 0.45 and 0.20 ppm downfield from the corresponding signals of **9**. Similarly, comparison of the ^{1}H-NMR spectral data on **10a** (**9b**) with those on **9a**, revealed that the signals due to H-1, H-9 and Me-8 of **10a** were shifted +0.45, +0.36 and +0.16 ppm, respectively.

Thus, L-side IV was deduced to be 8-acetyl shanzhiside methyl ester (**10**). This assignment was supported by the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **10** compared with that of **9**: the signals of **10** showed a downfield shift for C-8 ($\Delta\delta$=10.12 ppm)[14] and upfield shifts for C-9 ($\Delta\delta$=−3.01 ppm) and C-10 ($\Delta\delta$=−2.45 ppm), as seen in other C-8β-acetoxyl substituted/C-8β-hydroxyl substituted pairs of iridoid glucosides.[15]

Consequently, the stereochemistry of L-side IV was established as **10**, the same as that of

TABLE I. ^{13}C-NMR Spectral Data for **1** and **8**—**10** (50.10 MHz; TMS as an Internal Standard, CD_3OD, δ)

Carbon atom	**1**	**8**	**9**	**10**
1	94.08 d	94.96 d	94.87 d	95.71 d
3	138.55 d	152.95 d	152.74 d	153.56 d
4	113.15	111.45	111.31	109.88
5	74.69	37.84 d	41.46 d	42.28 d
6	74.49 d[a]	76.48 d	77.47 d	76.03 d
7	45.84 t	78.87 d[a]	47.92 t	47.62 t
8	87.36	78.69	79.61	89.73
9	56.62 d	49.35 d	51.86 d	48.85 d
10	22.16 q[b]	22.28 q	24.67 q	22.22 q
11	12.38 q	169.47	169.65	169.83
		51.97 q	51.77 q	51.77 q
Acetyl	172.89			173.81
	22.05 q[b]			22.22 q
1′	99.39 d	99.80 d	99.77 d	100.39 d
2′	74.20 d[a]	74.57 d	74.57 d	74.66 d
3′	77.61 d[c]	77.96 d[c]	77.93 d[c]	77.90 d[c]
4′	71.68 d	71.60 d	71.60 d	71.65 d
5′	78.02 d[c]	78.25 d[c]	78.25 d[c]	78.28 d[c]
6′	62.81 t	62.81 t	62.84 t	62.98 t

a—*c*) Assignments with the same sign may be interchanged in each column. Unmarked signals are singlets.

TABLE II. Coupling Constants for the Protons of the Cyclopentane Ring in **1**, **1a**, **8**, **8a**, **9**, **9a** and **10a**

Compound	$J_{1\alpha,9\beta}$	$J_{3,5\beta}$	$J_{5\beta,6\alpha}$	$J_{5\beta,9\beta}$	$J_{6\alpha,7\alpha}$	$J_{6\alpha,7\beta}$
Measured value						
1[a]	0.5	—	—	—	4.0	*d*)
1a[a]	0.5	—	—	—	4.5	0.5
8[a]	1.7	0.5	3.0	10.0	4.6	—
8a[b]	1.5	1.5	2.7	10.6	4.6	—
9[a]	1.5	0.5	*d*)	10.0	6.0	3.0
9a[b]	2.9	1.5	1.0	9.2	6.0	3.0
10a[b]	1.5	1.5	0.5	8.8	4.9	1.6
Calculated values[c]						
^{6}V	0.5		1.5	8.0	4.0	1.5
V_7 ($^6T_7$)	0.5		0.5	9.4	5.0	1.5
^{7}V	1.0		6.0	9.4	4.5	6.0
V_8 ($^7T_8$)	2.5		3.0	9.4	6.0	8.0
^{8}V	0.4		3.0	7.0	7.0	0.5
V_6 ($^7T_6$)	1.0		10.0	7.5	5.0	8.5
^{5}V	0.8		8.0	5.5	7.0	8.5

a) Run in CD_3OD. *b*) Run in $CDCl_3$. *c*) The values were calculated from the equation $J = 9.5\cos^2\theta - 0.5\cos\theta + 0.4$.[17] *d*) Obscured signal.

barlerin reported by Damtoft *et al.*[16] Its pentaacetate (**10a**) was identified as shanzhiside methyl ester hexaacetate (**9b**) by direct comparison with an authentic sample (mixed mp, IR, ^{1}H-NMR and thin layer chromatography (TLC)).

The preferred conformations of the cyclopentane ring of **1a**, **8a**, **9a** and **10a** in solution,

deduced from the extensive ^{1}H-NMR spectral analyses of these compounds, were as follows. As shown in Table II, comparison of the measured values of the coupling constants of the H-5, H-6, H-7 and H-9 signals of **1a**, **9a** and **10a** with the calculated values, and observations of the nuclear Overhauser effect (NOE) increments (**1a**, 10%; **9a**, 7%; **10a**, 13%) between H-1 and Me-8, indicated that the cyclopentane rings in **1a**, **9a** and **10a** exist as $V_7(^6T_7)$ or ^6V forms; that of **10a** was definitely in the ^6V form, since long-range coupling (W rule) was observed between protons βH-5 and βH-7 (see the chart). The cyclopentane ring in **8a** was deduced to be in the $V_8(^7T_8)$ form from the coupling constants (see Table II) of the H-5, H-6, H-7 and H-9 signals, as we reported previously,[2] and from the NOE increments (**8a**: 13, 8 and 13%) for αH-1, αH-6 and αH-7 from Me-8. Namely, the above results showed that the conformations of **1a**, **8a**, **9a** and **10a** are decided by the bulk of the substituent at C-7.

In this work, **9** and **10** were isolated for the first time from a Labiatae plant.

Experimental

All melting points are uncorrected. IR spectra were measured with a Hitachi IR-215 spectrometer. NMR spectra were measured on a JEOL JNN-PS-100 (^{1}H: 100 MHz) or an FX-100 (^{1}H: 200 MHz; ^{13}C: 50.10 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. TLC and preparative thin-layer chromatography (PTLC) were performed with Merck Kieselgel GF_{254} and PF_{254}, respectively. Spots were located under ultraviolet (UV) illumination or by spraying with 1%$Ce(SO_4)_2$ –10%H_2SO_4 and then heating.

Isolation of Iridoid Glucosides——Fresh whole plants (8 kg) of *L. amplexicaule* L. collected at the Botanic Garden of the Faculty of Pharmaceutical Sciences, Tokushima University, were extracted three times with MeOH. The MeOH extract was evaporated *in vacuo* to give a residue (413 g), part of which (207 g) was chromatographed over a celite 535 (100 g) column with water (2 l) as the eluent. The eluate was evaporated *in vacuo* to give a residue (160 g). A part (35 g) of the residue was chromatographed on active charcoal (150 g) with H_2O–MeOH (1 : 1) until fraction 3 (500 ml each), and then with MeOH. Fractions 5—9 (500 ml each) were concentrated to leave a residue (618 mg), which was then subjected to PTLC($CHCl_3$–MeOH–H_2O = 65 : 35 : 10, lower layer) to give crude L-side I (**1**) (80 mg, Rf = 0.52), L-side II (**8**) (207 mg, Rf = 0.20), L-side III (**9**) (59 mg, Rf = 0.37) and L-side IV (**10**) (49 mg, Rf = 0.59). The crude L-sides I—III (**1**, **8** and **9**) were purified further by PTLC($CHCl_3$–MeOH–AcOEt = 3 : 1 : 1, each developed five times) to obtain pure L-side I (**1**) (33.7 mg, Rf = 0.49), L-side II (**8**) (70 mg, Rf = 0.24) and L-side III (**9**) (34 mg, Rf = 0.44). The crude L-side IV (**9**) was purified by PTLC($CHCl_3$–MeOH–AcOEt = 4 : 1 : 2, developed seven times) to afford pure L-side IV (**10**) (33.7 mg, Rf = 0.33).

L-side I (1, Lamioside)——White powder, $[\alpha]_D^{25}$ –58.4° (c = 0.45, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1700 (C=O), 1660 (C=C). ^{1}H-NMR (CD_3OD) δ: 1.40 (3H, s, Me-8), 1.59 (3H, br s, Me-4), 1.98 (3H, s, AcO-8), 2.04 (2H, m, H_2-7), 2.78 (1H, br s, βH-9), 3.96 (1H, m, αH-6), 4.53 (1H, d, J = 8.0 Hz, H-1′), 5.96 (1H, br s, αH-1), 6.08 (1H, br s, H-3). ^{13}C-NMR: see Table I.

Acetylation of 1——**1** (7 mg) was acetylated with Ac_2O–pyridine (0.3 ml–0.3 ml) by the usual method to give **1a** (7 mg).

1a: Colorless needles (from EtOH), mp 204—206 °C, $[\alpha]_D^{26}$ –75.3° (c = 0.51, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1760, 1750, 1730, 1720, (C=O), 1660 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, s, Me-8), 1.65 (3H, br s, Me-4), 1.95—2.08 (AcO × 6), 3.02 (1H, br s, βH-9), 5.13 (1H, m, αH-6), 6.00 (2H, br s, H-1 and H-3). *Anal.* Calcd for $C_{28}H_{38}O_{16}$: C, 53.33; H, 6.07. Found: C, 53.43; H, 5.92.

L-side II (8, Lamalbid)——White powder, $[\alpha]_D^{26}$ –86.1° (c = 0.69, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1695 (C=O), 1635 (C=C). ^{1}H-NMR (CD_3OD) δ: 1.20 (3H, s, Me-8), 2.78 (1H, dd, J = 10.0, 1.7, βH-9), 2.93 (1H, ddd, J = 10.0, 3.0, 0.5 Hz, βH-5), 3.54 (1H, d, J = 4.6 Hz, αH-7), 3.72 (3H, s, MeOOC-4), 3.94 (1H, dd, J = 4.6, 3.0 Hz, αH-6), 4.60 (1H, d, J = 7.8 Hz, H-1′), 5.62 (1H, d, J = 1.7 Hz, αH-1), 7.40 (1H, d, J = 0.5 Hz, H-3). ^{13}C-NMR: see Table. I.

Acetylation of 8——**8** (7 mg) was acetylated with Ac_2O–pyridine (0.3 ml–0.4 ml) by the usual method to give a hexaacetate (**8a**, 7 mg).

8a: Colorless needles (from EtOH), mp 150—151 °C, $[\alpha]_D^{25}$ –57.9° (c = 0.35, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1770, 1720 (C=O), 1640 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, s, Me-8), 1.90—2.14 (AcO × 6), 2.88 (1H, dd, J = 10.6, 1.5 Hz, βH-9), 3.06 (1H, ddd, J = 10.6, 2.7, 1.5 Hz, βH-5), 3.67 (3H, s, MeOOC-4), 4.93 (1H, d, J = 4.6 Hz, αH-7), 5.26 (1H, dd, J = 4.6, 2.7 Hz, αH-6), 5.50 (1H, d, J = 1.5 Hz, αH-1), 7.30 (1H, d, J = 1.5 Hz, H-3). *Anal.* Calcd for $C_{25}H_{34}O_{16}$: C, 58.85; H, 5.80. Found: C, 50.25; H, 5.89.

L-side III (9, Shanzhiside Methyl Ester)——White powder, $[\alpha]_D^{26}$ –101.5° (c = 0.33, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (C=O), 1645 (C=O). ^{1}H-NMR (CD_3OD) δ: 1.28 (3H, s, Me-8), 1.92 (2H, m, H_2-7), 2.60 (1H, m, βH-9), 3.00 (1H, m, βH-5), 3.74 (3H, s, MeOOC-4), 4.85 (1H, d, J = 8.0 Hz, H-1′), 5.59 (1H, d, J = 1.5 Hz, αH-1), 7.42 (1H, s,

H-3). ^{13}C-NMR: see Table I.

Acetylation of 9——**9** (38 mg) was acetylated with Ac_2O–pyridine (0.3 ml–0.3 ml) at 50 °C for 4 h to give a pentaacetate (**9a**, 20 mg) and a hexaacetate (**9b**, 8.8 mg).

9a: Colorless needles (from EtOH), mp 177—179 °C, $[\alpha]_D^{26}$ −150.0° (c=0.30, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3540 (OH), 1740, 1730, 1700 (C=O), 1640 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, s, Me-8), 1.86 (1H, dd, J=14.9, 3.0 Hz, βH-7), 2.07 (1H, dd, J=14.9, 6.0 Hz, αH-7), 2.69 (1H, dd, J=9.2, 2.9 Hz, βH-9), 3.19 (1H, ddd, J=9.2, 1.5, 1.0 Hz, βH-5), 3.71 (3H, s, MeOOC-4), 4.86 (1H, d, J=8.1 Hz, H-1′), 5.27 (1H, dd, J=6.0, 3.0 Hz, αH-6), 5.43 (1H, d, J=2.9 Hz, αH-1), 7.38 (1H, d, J=1.5 Hz, H-3). *Anal.* Calcd for $C_{27}H_{36}O_{16}$: C, 52.59; H, 5.89. Found: C, 52.48; H, 6.03.

9b: Colorless needles (from EtOH), mp 185—187 °C, $[\alpha]_D^{26}$ −140.5° (c=0.67, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: no OH, 1760, 1740, 1710 (C=O), 1640 (C=C). ^{1}H-NMR ($CDCl_3$) δ: 1.49 (3H, s, Me-8), 1.97 (1H, dd, J=14.9, 4.9 Hz, αH-7), 2.33 (1H, ddd, J=14.9, 1.6, <0.5 Hz, βH-7), 3.05 (1H, dd, J=8.8, 1.5 Hz, βH-9), 3.12 (1H, dddd, J=8.8, 1.5, 0.5, <0.5, βH-5), 3.70 (3H, s, MeOOC-4), 4.85 (1H, d, J=8.3 Hz, H-1′), 5.30 (1H, ddd, J=4.9, 1.6, 0.5, αH-6), 5.88 (1H, d, J=1.5 Hz, αH-1), 7.39 (1H, d, J=1.5 Hz, H-3). *Anal.* Calcd for $C_{29}H_{38}O_{17}$: C, 52.88; H, 5.82. Found: C, 52.84; H, 5.69.

L-side IV (10, Barlerin)——White powder, $[\alpha]_D^{26}$ −81.0° (c=0.38, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1710 (C=O), 1640 (C=C). ^{1}H-NMR (CD_3OD) δ: 1.48 (3H, s, Me-8), 1.96 (3H, s, AcO-8), 2.12 (2H, m, H_2-7), 2.98 (2H, br s, βH-5 and βH-9), 3.68 (3H, s, MeOOC-4), 3.80 (1H, m, αH-6), 4.57 (1H, d, J=8.0 Hz, H-1′), 5.84 (1H, d, J=2.0 Hz, αH-1), 7.38 (1H, br s, H-3). ^{13}C-NMR: see Table I.

Acetylation of 10——**10** (33 mg) was acetylated with Ac_2O–pyridine (0.3 ml–0.3 ml) at 50 °C for 3 h to give a pentaacetate (**10a**, 19 mg) as colorless needles (from EtOH), mp 187—189 °C, $[\alpha]_D^{26}$ −147.4° (c=0.67, $CHCl_3$). This product was identical with an authentic sample of hexaacetylshanzhiside methyl ester (**9b**) on the basis of TLC, IR, ^{1}H-NMR comparisons and mixed mp determination.

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