



Secoiridoid glycosides from the flower buds of *Lonicera japonica*[☆]

Rie Kakuda, Mio Imai, Yasunori Yaoita, Koichi Machida, Masao Kikuchi *

Department of 2nd Analytical Chemistry, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai, Miyagi 981-8558, Japan

Received 22 December 1999; received in revised form 27 June 2000

Abstract

Two secoiridoid glycosides, loniceracetalides A and B, were isolated in very small amounts, together with 10 known iridoid glycosides, from the flower buds of *Lonicera japonica*. The structures of loniceracetalides A and B were determined by spectroscopic analysis. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Lonicera japonica*; Caprifoliaceae; Flower buds; Secoiridoid glycoside; Loniceracetalide A; Loniceracetalide B

1. Introduction

The flower buds of *Lonicera japonica* Thunb. are used in Chinese herbal medicine for their latent-heat-clearing, antipyretic, detoxicant and antiinflammatory actions (Chang and Hsu, 1992). The constituents of this plant have been previously investigated and shown to contain iridoid glucosides (Kawai et al., 1988; Mehrotra et al., 1988; Tomassini et al., 1995) and polyphenolic compounds (Chang and Hsu, 1992). As a part of our continuing study of the constituents of *Lonicera* species, we now report the isolation and structural elucidation of two secoiridoid glycosides, loniceracetalide A (**1**) and loniceracetalide B (**2**), together with 10 known iridoid glycosides (**3**–**12**) from the flower buds of *L. japonica*.

2. Results and discussion

Known compounds **3**–**7** were identified as loganin (**3**) (Machida et al., 1995), secologanin (**4**) (Machida et al., 1995), secologanin dimethyl acetal (**5**) (Machida et al., 1995), sweroside (**6**) (Machida et al., 1995) and secologanoside 7-methyl ester (**7**) (Sugiyama et al., 1993) by direct comparison with authentic samples. Compounds **8**–**12** were identified as kingiside (**8**) (Souzu and Mitsuhashi, 1969), morroniside (**9**) (Souzu and Mitsuhashi,

1969), 8-epiloganin (**10**) (Bianco and Passacantilli, 1981), vogeloside (**11**) (Kawai et al., 1988) and epivogeloside (**12**) (Kawai et al., 1988) on the basis of their spectral and physical data. Among these, compounds **5**, **11** and **12** may be artifacts formed from compound **4** during the extraction and isolation processes (Tomassini et al., 1995). The presence of compound **10** in Caprifoliaceae is unexpected, since compounds with the 8 α -methyl group have so far been restricted to the plants of Scrophulariaceae (Bianco and Passacantilli, 1981) and Loasaceae (Rodriguez et al., 1997) respectively.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{20} -106.1^\circ$. Its UV spectrum showed an absorption at 232 nm. The FABMS of **1** showed two quasimolecular ions at m/z 461 ($[M+H]^+$) and m/z 483 ($[M+Na]^+$). The 1H NMR (Experimental) and ^{13}C NMR (Table 1) spectra were very similar to those of **5**, but lacked the signals from two methoxyl groups at C-7 of **5**, and instead showed signals due to two secondary methyl groups [δ_H 1.097 (3H, d , $J=6.2$ Hz, H-1'' or H-4''), 1.099 (3H, d , $J=6.2$ Hz, H-1'' or H-4''); δ_C 14.6 (C-1'', C-4'')] and two oxygenated methines [δ_H 4.17 (1H, q , $J=6.2$ Hz, H-2'' or H-3''), 4.21 (1H, q , $J=6.2$ Hz, H-2'' or H-3''); δ_C 75.2, 75.3 (C-2'', C-3'')]. Detailed analysis of the 1H – 1H COSY spectrum implied connectivities for H-1''–H-2'', H-3''–H-4'' and H-6–H-7. In the HMBC spectrum, two secondary methyl groups (H-1'' and H-4'') showed connectivity to C-2'' and C-3'' and the methylene protons (H-6) to C-7. These spectral data suggested that **1** has a dimethylethylene acetal group instead of the dimethyl acetal group in **5**. The stereochemistry of this acetal group was determined by analysis

[☆] Part 14 in the series 'Studies on the constituents of *Lonicera* species'. For Part 13: Kikuchi, M., Kawarada, N., Yaoita, Y., 1999. Chemical & Pharmaceutical Bulletin, 47, 663.

* Corresponding author.

Table 1
 ^{13}C NMR spectral data (150 MHz) of compounds **1**, **2** and **5** (in CD_3OD)

C	1	2	5
1	97.8	97.7	97.9
3	153.1	153.2	153.3
4	111.7	111.5	111.7
5	29.5	29.5	29.4
6	35.5	35.4	33.3
7	102.6	103.2	104.5
8	135.8	135.7	135.9
9	45.3	45.2	45.4
10	119.7	120.0	119.8
11	169.2	169.2	169.2
COOMe	51.7	51.7	51.7
Glc-1	100.1	100.1	100.2
Glc-2	74.7	74.6	74.7
Glc-3	78.0	78.0	78.1
Glc-4	71.5	71.5	71.6
Glc-5	78.4	78.4	78.5
Glc-6	62.7	62.7	62.8
1''	14.6 ^a	15.7 ^b	
2''	75.2 ^b	75.7 ^b	
3''	75.3 ^b	75.9 ^b	
4''	14.6 ^a	15.8 ^b	
OMe			52.6 54.0

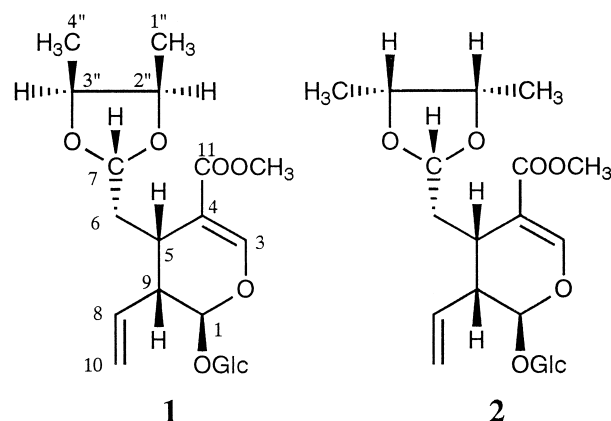
^a Signals overlap.

^b Assignments in same vertical column may be interchanged.

of the NOESY spectrum, where correlations from H-7 with H-5, H-9, H-1'' and H-4'' suggested that these protons were present on the same side of the molecule. On the basis of the above data, the structure of loniceracetalide A (**1**) was established as depicted in the formula.

Compound **2** was isolated as an amorphous powder, $[\alpha]_{\text{D}}^{20} -115.0^\circ$. The FABMS of **2** showed two quasimolecular ions at m/z 461 ($[\text{M} + \text{H}]^+$) and m/z 483 ($[\text{M} + \text{Na}]^+$). Comparison of the ^1H and ^{13}C NMR spectral data with those of **1** revealed good agreement, except for the signals due to the dimethylethylene acetal moiety. In the NOESY spectrum, the NOE correlation from H-7 with H-5, H-9, H-2'' and H-3'' indicated that these protons were present on the same side of the molecule. Thus, **2** was the diastereomer of **1**. From the above data, the structure of loniceracetalide B (**2**) was determined as depicted in the formula.

There was a possibility that compounds **1** and **2** might be artifacts arising from action of secologanin and *meso*-butane-2,3-diol, which was considered as a possible impurity in the solvent(s) used the extraction and isolation procedures. In order to exclude the above hypothesis, the solvents used in this study, methanol (3 l), chloroform (1 l), diethyl ether (1 l) and ethyl acetate (1 l) were distilled at atmospheric pressure, respectively and each residue was checked by ^1H NMR spectra (600 MHz). No *meso*-butane-2,3-diol was detected in each residue. Thus **1** and **2** are considered to be naturally occurring compounds. Compounds **1** and **2** are similar to 7-dioxola-



nylsecologanin, which was isolated from the aerial parts of *Gentiana verna* (Mpondo and Garcia, 1990), and are the first secoiridoid glycosides with a dimethylethylene acetal group from a natural source.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded with a JEOL JNM-LA 600 spectrometer (600 and 150 MHz, respectively). Chemical shifts are given relative to TMS as int. standard. UV spectra were recorded with a Beckman DU-64 spectrophotometer. Optical rotations were determined using a JASCO DIP-360 digital polarimeter. CC was carried out on Kieselgel 60 (230–400 mesh, Merck). HPLC was carried out on a Tosoh HPLC system (pump, CCPS; detector, UV-8020).

3.2. Plant material

The dried flower buds of *L. japonica* (from Shandong, China) were purchased from Uchida Wakanyaku Co., Ltd., Tokyo, Japan in 1998. A voucher specimen (No. 7) is deposited in the laboratory of M. Kikuchi.

3.3. Extraction and isolation

The dried flower buds of *L. japonica* (2.0 kg) were extracted with MeOH at room temp. The MeOH extract was successively extracted with CHCl_3 , Et_2O , EtOAc and *n*-BuOH. The EtOAc-soluble fr. was concd. under red. pres. to afford a residue (17.2 g). A part of this residue (10.5 g) was subjected to silica gel column chromatography using CHCl_3 –MeOH– H_2O (30:10:1) as eluant, with the eluate sepd. into 30 frs. (frs. 1–30). Frs. 5–7 were purified by prep. HPLC [Column, TSK gel ODS-120T (7.8 mm i.d. \times 30 cm); mobile phase, MeOH– H_2O (1:1); flow rate, 1.0 ml min^{-1} ; UV detector, 230 nm] to give **1** (1.1 mg), **2** (3.4 mg), **4** (3.2 mg), **5** (5.5 mg) and **7** (2.1 mg).

The *n*-BuOH-soluble fr. was concd. under red. pres. to afford a residue (72.8 g). A part of this residue (15.0 g) was applied to a silica gel column using CHCl₃–MeOH–H₂O (30:10:1), and the eluate was sepd. into 50 frs. (frs. 1–50). Frs. 13–20 were purified by prep. HPLC [Column, TSK gel ODS-120T (7.8 mm i.d.×30 cm); mobile phase, MeOH–H₂O (1:1); flow rate, 1.0 ml min^{−1}; UV detector, 230 nm] to give **3** (3.0 mg), **4** (1.2 mg), **5** (2.2 mg), **6** (2.5 mg), **8** (1.6 mg), **9** (1.5 mg), **10** (1.2 mg), **11** (8.2 mg) and **12** (2.5 mg).

3.4. *Loniceracetalide A* (**1**)

Amorphous powder; $[\alpha]_D^{20}$ −106.1° (MeOH; *c* 0.1); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 232 (4.07); ¹H NMR spectral data (CD₃OD): δ 1.097 (3H, *d*, *J* = 6.2 Hz, H-1'' or H-4''), 1.099 (3H, *d*, *J* = 6.2 Hz, H-1'' or H-4''), 1.67 (1H, *ddd*, *J* = 13.6, 8.1, 4.8 Hz, H_A-6), 1.96 (1H, *ddd*, *J* = 13.6, 6.2, 6.2 Hz, H_B-6), 2.74 (1H, *m*, H-9), 2.97 (1H, *m*, H-5), 3.19 (1H, *dd*, *J* = 9.2, 8.1 Hz, glc-H-2), 3.28–3.37 (3H, *m*, glc-H-3, glc-H-4, glc-H-5), 3.66 (1H, *dd*, *J* = 11.7, 6.2 Hz, glc-H_A-6), 3.68 (3H, *s*, COOCH₃), 3.89 (1H, *dd*, *J* = 11.7, 1.8 Hz, glc-H_B-6), 4.17 (1H, *q*, *J* = 6.2 Hz, H-2'' or H-3''), 4.21 (1H, *q*, *J* = 6.2 Hz, H-2'' or H-3''), 4.66 (1H, *d*, *J* = 8.1 Hz, glc-H-1), 5.252 (1H, *dd*, *J* = 10.3, 1.1 Hz, H_A-10), 5.253 (1H, *dd*, *J* = 6.2, 4.8 Hz, H-7), 5.28 (1H, *dd*, *J* = 17.2, 1.1 Hz, H_B-10), 5.51 (1H, *d*, *J* = 5.1 Hz, H-1), 5.72 (1H, *ddd*, *J* = 17.2, 10.3, 7.0 Hz, H-8), 7.41 (1H, *d*, *J* = 1.5 Hz, H-3); ¹³C NMR spectral data: Table 1; FABMS *m/z*: 461 [M + H]⁺, 483 [M + Na]⁺.

3.5. *Loniceracetalide B* (**2**)

Amorphous powder; $[\alpha]_D^{20}$ −115.0° (MeOH; *c* 0.3); UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 233 (4.04); ¹H NMR spectral data (CD₃OD): δ 1.10 (3H, *d*, *J* = 5.9 Hz, H-1'' or H-4''), 1.11 (3H, *d*, *J* = 5.9 Hz, H-1'' or H-4''), 1.77 (1H, *ddd*,

J = 13.6, 8.4, 4.4 Hz, H_A-6), 2.06 (1H, *ddd*, *J* = 13.6, 8.1, 5.9 Hz, H_B-6), 2.72 (1H, *m*, H-9), 3.00 (1H, *m*, H-5), 3.19 (1H, *dd*, *J* = 9.2, 8.1 Hz, glc-H-2), 3.24–3.37 (3H, *m*, glc-H-3, glc-H-4, glc-H-5), 3.67 (1H, *dd*, *J* = 11.7, 6.2 Hz, glc-H_A-6), 3.68 (3H, *s*, COOCH₃), 3.89 (1H, *dd*, *J* = 11.7, 1.8 Hz, glc-H_B-6), 4.06 (1H, *q*, *J* = 5.9 Hz, H-2'' or H-3''), 4.08 (1H, *q*, *J* = 5.9 Hz, H-2'' or H-3''), 4.67 (1H, *d*, *J* = 8.1 Hz, glc-H-1), 4.92 (1H, *dd*, *J* = 5.9, 4.4 Hz, H-7), 5.25 (1H, *dd*, *J* = 10.3, 1.1 Hz, H_A-10), 5.28 (1H, *br d*, *J* = 17.2 Hz, H_B-10), 5.51 (1H, *d*, *J* = 5.1 Hz, H-1), 5.72 (1H, *ddd*, *J* = 17.2, 10.3, 7.0 Hz, H-8), 7.41 (1H, *d*, *J* = 1.1 Hz, H-3); ¹³C NMR spectral data: Table 1; FABMS *m/z*: 461 [M + H]⁺, 483 [M + Na]⁺.

Acknowledgements

We are grateful to Mr. S. Sato and Mr. T. Matsuki of this University for providing the mass and NMR spectra.

References

- Bianco, A., Passacantilli, P., 1981. *Phytochemistry* 20, 1873.
- Chang, W.-C., Hsu, F.-L., 1992. *Prostaglandins Leukotrienes and Essential Fatty Acids* 45, 307.
- Kawai, H., Kuroyanagi, M., Ueno, A., 1988. *Chemical & Pharmaceutical Bulletin* 36, 3664.
- Machida, K., Asano, J., Kikuchi, M., 1995. *Phytochemistry* 39, 111.
- Mehrotra, R., Singh, C., Popli, S.P., 1988. *Journal of Natural Products* 51, 319.
- Mpondo, E.M., Garcia, J., 1990. *Planta Medica* 56, 125.
- Rodriguez, V., Schripsema, J., Jensen, S.R., 1997. *Phytochemistry* 45, 1427.
- Souzu, I., Mitsunashi, H., 1969. *Tetrahedron Letters*, 2725.
- Sugiyama, M., Machida, K., Matsuda, N., Kikuchi, M., 1993. *Phytochemistry* 34, 1169.
- Tomassini, L., Cometa, M.F., Serafini, M., Nicoletti, M., 1995. *Journal of Natural Products* 58, 1756.