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Phytochemical Studies on Lonicerae Flos (1) – Isolation of Iridoid Glycosides and other Constituents

Eun Ju Lee, Joo Young Lee, Ju Sun Kim, and Sam Sik Kang*

Natural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Abstract – From the polar fractions of a 70% EtOH extract of the flower buds of *Lonicera japonica* (Caprifoliaceae), ten constituents were isolated and identified as iridoid glycosides 7-dehydrologanin (7-ketologanin, **2**), secologanin dimethyl acetal (**3**), (*E*)-aldosecologanin (centauroside, **5**), dimethyl secologanoside (**6**), secoxyloganin (**7**) and epivogeloside (**8**). Other identified constituents were 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E/Z*)-2-[(2*R*)-2-hydroxy(docosanoyl, tricosanoyl, tetracosanoyl, pentacosanoyl)amino]-8-octadecene-1,3,4-triol (**1**), uracil (**4**), D-mannitol (**9**), and sucrose (**10**). Among them, **1**, **2**, **4**, and **10** were isolated for the first time from this plant.
Keywords – *Lonicera japonica*, Caprifoliaceae, iridoid glycosides, cerebroside, isolation and identification

Introduction

Lonicera is an important genera in the Caprifoliaceae family. Some *Lonicera* spp. have been used in Chinese herbal medicine for the treatment of acute fever, headache, respiratory infection, pharyngodynia, and some epidemic diseases, for centuries (Shi *et al*., 1999). Among them, the flower buds of *Lonicera japonica* Thunb., medicinally knowns as Lonicerae Flos have been reported to act as anti-inflammatory (Moon *et al*., 1999; Son *et al*., 2006; Yoo *et al*., 2008), antioxidant (Choi *et al*., 2007), and antibacterial agents in herbal medicine. Numerous chemical constitutions such as iridoids (Kawai *et al*., 1988; Kakuda *et al*., 2000), flavonoids (Son *et al*., 1992; Son *et al*., 1994a), saponins (Kwak *et al*., 2003; Lin *et al*., 2008; Son *et al*., 1994b), polyphenols (Chang & Hsu, 1992), and cerebrosides (Kumar *et al*., 2006) have been reported from this plant. In spite of the various research on *L. japonica*, investigations on the chemical constituents were carried out on its polar fraction extracts. This paper elucidates the structure of six iridoid glycosides, together with cerebroside, uracil, D-mannitol, and sucrose from the flower buds of *L. japonica* on the basis of various spectroscopic data.

Experimental

General – The optical rotation was measured on a Jasco P-1020 polarimeter. IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. UV spectra were measured on a Hitachi JP/U-3010 spectrophotometer. FAB-MS was obtained on a VG-VSEQ spectrometer. 1D and 2D NMR spectra were obtained on a Varian Gemini 2000 (300 MHz) and Bruker Avance 400, 500 (400, 500 MHz), and the chemical shifts were referenced to TMS. TLC was performed on silica gel 60 F254 (Merck, art. no. 5715), C18 (Merck, art. no. 5685) and cellulose plates (Merck, art. no. 5716). Spots on the plates were observed under UV light and visualized by spraying them with 20% H_2SO_4 in H_2O (v/v), followed by heating.

Plant Material – Flower buds of *L. japonica* were purchased from China in April 2007, and authenticated by Professor Lee, J.-H. (College of Oriental Medicine, Dongguk University). A voucher specimen (LJH2007-4) was deposited in the herbarium of the College of Oriental Medicine, Dongguk University.

Extraction and Isolation – Powdered flower buds of *L. japonica* (19.4 kg) were refluxed with 70% EtOH for 3 h at 70 – 80 °C. The 70% EtOH extract was evaporated to dryness under reduced pressure and then successively partitioned between H_2O and hexane (389.6 g), CH_2Cl_2 (271.1 g), EtOAc (375.7 g) and then BuOH (1767.5 g), respectively. The EtOAc fraction (187.6 g) was fractionated by column chromatography over silica gel with CH_2Cl_2-MeOH-H_2O (7 : 0.8 : 0.5 → 13 : 7 : 2) to yield 16 subfractions (Fr. E–01 – Fr. E–16). Fr. E-9 (29.3 g) was chromatographed on a silica gel column with hexane-EtOAc and then with EtOAc-MeOH (gradient) to afford

*Author for correspondence
Tel: +82-2-880-2481; E-mail: sskang@snu.ac.kr

subfraction E-9-38, which was then recrystallized from CH_2Cl_2/MeOH to yield **1** (3 mg). Subfraction E-9-3435 (8.09 g) was further chromatographed on a silica gel column with hexane-EtOAc (5 : 8 → 1 : 10) and then EtOAc saturated with H_2O-MeOH (gradient) to give **2** (3 mg) and **3** (181 mg), respectively. Subfraction E-9-5254 (1.37 g) was chromatographed on a silica gel column with CH_2Cl_2-MeOH-H_2O (7 : 1 : 0.5 → 7 : 2 : 0.5) to yield **4** (6 mg). Fr. E-12 (37.2 g) was purified on a silica gel column with EtOAc-MeOH-H_2O (100 : 4 : 3) to yield Fr. E-12-2631 (4.94 g), which was further chromatographed on a silica gel column with EtOAc-MeOH-H_2O (100 : 8 : 6 → 100 : 16.5 : 13.5) to afford subfraction E-12-2631-2 (2.77 g), which was further purified on an RP-18 column with 50% MeOH to give **5** (70 mg). The BuOH fraction (612.2 g) was fractionated by silica gel column chromatography with CH_2Cl_2-MeOH (gradient) to yield 20 subfractions (Fr. B–01 – Fr. B-20). Fr. B-12 (36.9 g) was chromatographed on a silica gel column with EtOAc saturated with H_2O-MeOH (gradient) to afford subfraction B-12-1317 (1.5 g), which was then separated on a Sephadex LH-20 column to yield **6** (63 mg). Subfraction B-12-5458 (4.29 g) was chromatographed on a silica gel column with $CHCl_3$-MeOH (10 : 1) to afford subfraction B-12-5458-4344 (800 mg), which was further purified on an RP-18 column with 50% MeOH to give **7** (30 mg). Fr. B-14 (28.5 g) was chromatographed on a silica gel column with CH_2Cl_2-MeOH-H_2O (7 : 0.5 : 0.5 → 7 : 3 : 1) followed by chromatography on silica gel column with EtOAc saturated with H_2O-MeOH (gradient) to yield subfraction B-14-2832 (1.24 g), which was then chromatographed on a silica gel column with hexane-EtOAc (1 : 10) to yield **8** (129 mg). Fr. B-18 was recrystallized from CH_2Cl_2/MeOH to give **9** (1,068 mg) and **10** (10 mg), respectively.

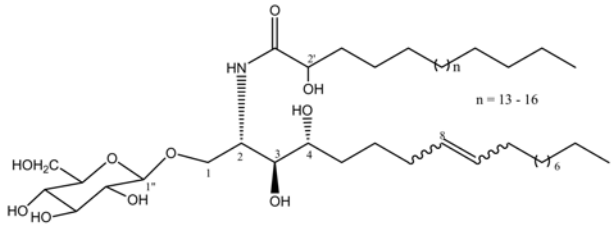
Cerebroside (1) – Amorphous white powder. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 0.83 (6H, m, 2 × CH_3), 1.20 – 1.27 [br s, $(CH_2)_n$], 3.85 (1H, m, H-5"), 4.01 (1H, t, J = 8.0 Hz, H-2"), 4.19 (1H, t, J = 8.7 Hz, H-4), 4.21 (1H, t, J = 8.8 Hz, H-4"), 4.22 (1H, t, J = 8.8 Hz, H-3"), 4.29 (1H, dd, J = 5.1, 10.4 Hz, H-3), 4.34 (1H, dd, J = 5.1, 11.0 Hz, H-6"a), 4.48 (1H, br d, J = 11.0 Hz, H-6"b), 4.51 (1H, dd, J = 4.1, 11.5 Hz, H-1a), 4.57 (1H, m, H-2'), 4.72 (1H, dd, J = 6.9, 11.5 Hz, H-1b), 4.94 (1H, d, J = 7.7 Hz, H-1"), 5.31 (1H, m, H-2), 5.40 – 5.53 (2H, m, H-8, 9), 8.60 (1H, d, J = 9.2 Hz, NHCO); ^{13}C-NMR (pyridine-d_5, 125 MHz) δ: 70.4 (C-1), 51.6 (C-2), 75.8 (C-3), 72.3 (C-4), 130.3, 130.1, 130.7, 130.7 (CH=CH), 175.6 (C-1'), 72.3 (C-2'), 35.5 (C-3'), 105.5 (C-1"), 75.1 (C-2"), 78.5 (C-3"), 71.2 (C-4"), 78.3 (C-5"), 62.4 (C-6"), 14.2 (CH_3), 22.9, 25.8, 26.6, 26.7, 26.9, 27.5, 27.8, 29.5, 29.6, 29.9, 30.0, 30.3, 32.1, 32.9, 33.3, 33.8, 33.9; FABMS *m/z* 838 $[M + Na]^+$ (2-hydroxybehenic acid), 852 $[M + Na]^+$ (2-hydroxytricosanoic acid), 866 $[M + Na]^+$ (2-hydroxylignoceric acid), 880 $[M + Na]^+$ (2-hydroxypentacosanoic acid).

7-Dehydrologanin (7-ketologanin, 2) – Amorphous white powder. $[\alpha]_D^{26} = -58.0°$ (*c* 0.13, MeOH). IR ν_{max} (KBr) 3432 (OH), 1748 (5-membered ring C=O), 1684, 1643 (α,β-unsat. C=O), 1451, 1299 (ester), 1076 (glycosidic C-O), 889, 843 cm^{-1}; UV λ_{max} (log ε) (MeOH) 236 (sh, 4.20) nm; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 0.93 (3H, d, J = 7.6 Hz, 10-CH_3), 2.16 (1H, dq, J = 7.1, 10.5 Hz, H-8), 2.38 (1H, ddd, J = 2.7, 7.3, 10.5 Hz, H-9), 2.64 (1H, dd, J = 8.3, 18.9 Hz, H-6b), 2.77 (1H, br d, J = 18.9 Hz, H-6a), 3.37 (1H, br t, J = 7.2 Hz, H-5), 3.49 (3H, s, $COOCH_3$), 4.00 (1H, t, J = 7.8 Hz, H-5'), 4.07 (1H, t, J = 8.0 Hz, H-2'), 4.26 – 4.29 (2H, m, H-3', 4'), 4.36 (1H, dd, J = 5.3, 11.8 Hz, H-6'a), 4.54 (1H, br d, J = 11.8 Hz, H-6'b), 5.35 (1H, d, J = 7.9 Hz, H-1'), 5.92 (1H, d, J = 2.6 Hz, H-1), 7.69 (1H, s, H-3); ^{13}C-NMR (pyridine-d_5, 100 MHz) δ: see Table 1; FABMS *m/z* 411 $[M + Na]^+$, 389 $[M + H]^+$, 227 $[(M + H) - 162]^+$.

Secologanin dimethyl acetal (3) – Amorphous white powder. $[\alpha]_D^{28} = -102.7°$ (*c* 0.45, MeOH). IR ν_{max} (KBr) 3408 (OH), 1705 (ester), 1631 (α,β-unsat. C=O), 1439 (CH_2), 1389 (CH_3), 1287 (ester), 1076 (glycosidic C-O), 872, 768 cm^{-1}; UV λ_{max} (log ε) (MeOH) 232 (4.10) nm; ^{1}H-NMR (CD_3OD, 500 MHz) δ: 1.61 (1H, ddd, J = 4.6, 8.1, 14.0 Hz, H-6a), 2.07 (1H, dt, J = 6.6, 14.0 Hz, H-6b), 2.66 (1H, dt, J = 5.4, 8.8 Hz, H-9), 2.90 (1H, br dd, J = 6.0, 12.7 Hz, H-5), 3.27, 3.29 (3H each, s, 2 × OCH_3), 3.65 (1H, dd, J = 5.8, 11.9 Hz, H-6'a), 3.69 (3H, s, $COOCH_3$), 3.89 (1H, dd, J = 1.8, 11.9 Hz, H-6'b), 4.48 (1H, dd, J = 4.5, 7.1, H-7), 4.66 (1H, d, J = 8.0 Hz, H-1'), 5.26 (1H, d, J = 10.5 Hz, H-10a), 5.30 (1H, d, J = 17.3 Hz, H-10b), 5.50 (1H, d, J = 7.3 Hz, H-1), 5.72 (1H, ddd, J = 9.1, 10.2, 17.3 Hz, H-8), 7.42 (1H, s, H-3); ^{13}C-NMR (CD_3OD, 75.5 MHz) δ: see Table 1; FABMS *m/z* 457 $[M + Na]^+$, 403 $[(M + H) - CH_3OH]^+$, 241 $[(M + H) - (CH_3OH + 162)]^+$.

Uracil (4) – Amorphous white powder. IR ν_{max} (KBr) 1715 (CO), 1650 (CONH), 1451, 1418, 1233 (C-N), 821 cm^{-1}; UV λ_{max} (log ε) (MeOH) 258 (3.50) nm; ^{1}H-NMR (pyridine-d_5, 300 MHz) δ: 5.80 (1H, d, J = 7.5 Hz, H-5), 7.50 (1H, d, J = 7.5 Hz, H-6); ^{13}C-NMR (pyridine-d_5, 75.5 MHz) δ: 153.2 (C-2), 165.7 (C-4), 101.2 (C-5), 142.1 (C-6); EIMS *m/z* (rel. int., %) 112 $[M]^+$ (100), 69 $[M - HNCO]^+$ (47).

(*E*)-Aldosecologanin (centauroside, 5) – Amorphous white powder. $[\alpha]_D^{28} = -155.9°$ (*c* 0.22, MeOH). IR ν_{max} (KBr) 3409 (OH), 1691, 1633 (α,β-unsat. C=O), 1440,



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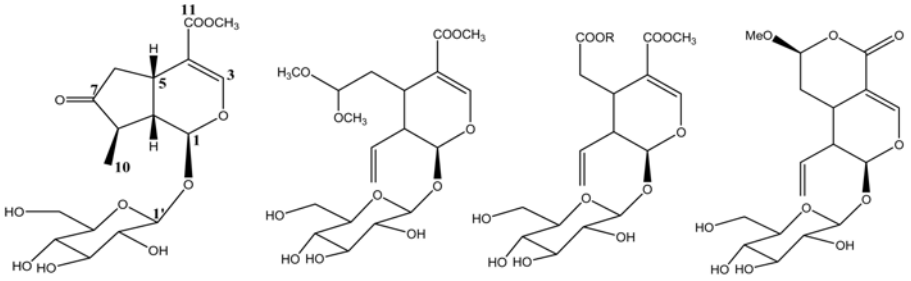
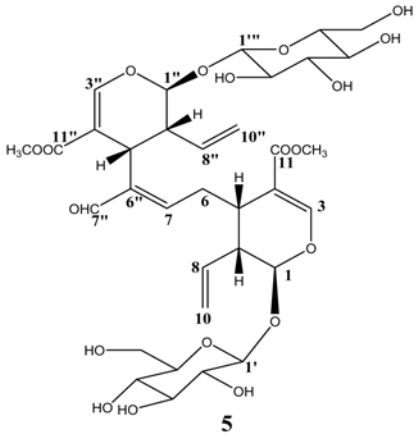


2 **3** **6** R=CH_3 **8**

7 R=H



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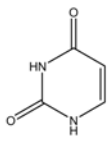

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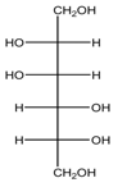

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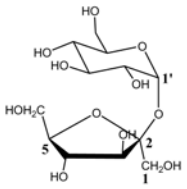

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1300 (ester), 1076 (glycosidic C-O), 930 (CH=CH_2), 767 cm^{-1}; UV λ_{max} (log ε) (MeOH) 232 (sh, 4.30), 252 (sh, 4.10), 282 (3.80) nm; ^{1}H-NMR (CD_3OD, 300 MHz) δ: 2.44 (1H, m, H-6a), 2.59 (1H, m, H-9"), 2.78 (1H, m, H-9), 3.09 (2H, m, H-5, 6b), 3.18 (1H, dd, *J* = 7.8, 9.0 Hz, H-2'), 3.59 (3H, s, 11"-$COOCH_3$), 3.72 (3H, s, 11-$COOCH_3$), 3.82 (1H, br d, *J* = 10.8 Hz, H-6'"), 3.89 (1H, dd, *J* = 2.1, 12.0 Hz, H-6'), 4.04 (1H, m, H-5"), 4.68 (1H, d, *J* = 7.8 Hz, H-1'"), 4.69 (1H, d, *J* = 8.1 Hz, H-1'), 5.02 (1H, br d, *J* = 8.7 Hz, H-10"a), 5.06 (1H, br d, *J* = 15.8 Hz, H-10"b), 5.29 (1H, br d, *J* = 11.4 Hz, H-10a), 5.35 (1H, br d, *J* = 17.4 Hz, H-10b), 5.49 (1H, d, *J* = 3.9 Hz, H-1"), 5.57 (1H, d, *J* = 4.8 Hz, H-1), 5.59 (1H, dd, *J* = 9.9, 17.1 Hz, H-8"), 5.79 (1H, dd, *J* = 8.4, 16.8 Hz, H-8), 6.71 (1H, t, *J* = 6.9 Hz, H-7), 7.47 (1H, s, H-3"), 7.54 (1H, s, H-3), 9.21 (1H, s, H-7"); ^{13}C-NMR (CD_3OD, 75.5 MHz) δ: see Table 1; FABMS *m/z* 781 $[M + Na]^+$, 759 $[M + H]^+$, 619 $[(M + Na) - 162]^+$, 597 $[(M + H) - 162]^+$, 435 $[(M + H) - (2 \times 162)]^+$.

Dimethyl secologanoside (6) – Amorphous white powder. $[\alpha]_D^{26} = -123.7°$ (*c* 0.14, MeOH). IR ν_{max} (KBr) 3408 (OH), 2952, 1707 (ester), 1630 (α,β-unsat. C=O),

1438 (CH_2), 1372 (CH_3), 1287 (ester), 1186, 1076 (glycosidic C-O), 948, 769 cm^{-1}; UV λ_{max} (log ε) (MeOH) 232 (4.03) nm; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 2.33 (1H, dd, *J* = 8.4, 16.2 Hz, H-6a), 2.75 (1H, m, H-9), 2.85 (1H, dd, *J* = 5.6, 16.2 Hz, H-6b), 3.18 – 3.22 (1H, m, H-5), 3.64, 3.66 (3H each, s, 2 × $COOCH_3$), 3.65 – 3.68 (1H, overlap, H-6'a), 3.88 (1H, dd, *J* = 1.5, 12.2 Hz, H-6'b), 4.65 (1H, d, *J* = 7.6 Hz, H-1'), 5.21 (1H, br d, *J* = 10.1 Hz, H-10a), 5.25 (1H, d, *J* = 17.3 Hz, H-10b), 5.47 (1H, d, *J* = 4.3 Hz, H-1), 5.62 (1H, dt, *J* = 9.8, 17.1 Hz, H-8), 7.47 (1H, d, *J* = 1.6 Hz, H-3); ^{13}C-NMR (CD_3OD, 75.5 MHz) δ: see Table 1; FABMS *m/z* 441 $[M + Na]^+$, 419 $[M + H]^+$, 257 $[(M + H) - 162]^+$.

Secoxyloganin (7) – Amorphous white powder. $[\alpha]_D^{26}$ = –110.6° (*c* 0.12, MeOH). IR ν_{max} (KBr) 3375 (OH), 2924, 1695 (ester), 1632 (α,β-unsat. C=O), 1568, 1403, 1294 (ester), 1076 (glycosidic C-O), 949, 772 cm^{-1}; UV λ_{max} (log ε) (MeOH) 230 (3.98) nm; ^{1}H-NMR (CD_3OD, 500 MHz) δ: 2.10 (1H, dd, *J* = 9.6, 15.7 Hz, H-6a), 2.81 – 2.84 (2H, m, H-6b, 9), 3.20 (1H, t, *J* = 9.1 Hz, H-2'), 3.27 – 3.29 (3H, m, H-5, 4', 5'), 3.36 (1H, t, *J* = 8.8 Hz, H-3'), 3.66 (3H, s, $COOCH_3$), 3.64 – 3.65 (1H, m, H-6'a), 3.87 (1H, dd, *J* = 1.4, 11.2 Hz, H-6'b), 4.63 (1H, d, *J* = 7.9 Hz, H-1'), 5.18 (1H, dd, *J* = 1.6, 10.5 Hz, H-10a), 5.25 (1H, d, *J* = 17.2 Hz, H-10b), 5.47 (1H, d, *J* = 3.9 Hz, H-1), 5.66 (1H, dt, *J* = 9.9, 17.1 Hz, H-8), 7.40 (1H, d, *J* = 1.6 Hz, H-3); ^{13}C-NMR (CD_3OD, 125.5 MHz) δ: see Table 1; FABMS *m/z* 427 $[M + Na]^+$.

Epivogeloside (8) – Amorphous white powder. $[\alpha]_D^{28}$ = –86.4° (*c* 0.12, MeOH). IR ν_{max} (KBr) 3408 (OH), 1699 (δ-lactone), 1618 (α,β-unsat. C=O), 1392, 1266, 1075, 1039 (glycosidic C-O), 905 (CH=CH_2), 838, 757 cm^{-1}; UV λ_{max} (log ε) (MeOH) 234 (4.19) nm; ^{1}H-NMR (CD_3OD, 500 MHz) δ: 1.71 (1H, ddd, *J* = 2.9, 13.7, 13.7 Hz, H-6*ax*), 1.86 (1H, ddd, *J* = 1.6, 5.0, 13.7 Hz, H-6*eq*), 2.64 (1H, m, H-9), 3.16 – 3.39 (5H, m, H-5, 2' – 5'), 3.51 (3H, s, $COOCH_3$), 3.66 (1H, dd, *J* = 5.7, 12.0 Hz, H-6'a), 3.88 (1H, dd, *J* = 2.0, 12.0 Hz, H-6'b), 4.68 (1H, d, *J* = 7.9 Hz, H-1'), 5.27 (1H, dd, *J* = 1.8, 10.2 Hz, H-10a), 5.29 (1H, dd, *J* = 1.8, 17.1 Hz, H-10b), 5.33 (1H, dd, *J* = 1.6, 2.9 Hz, H-7), 5.51 (1H, m, H-8), 5.55 (1H, d, *J* = 1.7 Hz, H-1), 7.61 (1H, d, *J* = 2.4 Hz, H-3); ^{13}C-NMR (CD_3OD, 125.5 MHz) δ: see Table 1; FABMS *m/z* 411 $[M + Na]^+$, 389 $[M + H]^+$.

D-Mannitol (9) – Amorphous white powder. $[\alpha]_D^{23}$ = +4.6° (*c* 0.1, MeOH). ^{1}H-NMR [D_2O (ref.: δ 4.80), 400 MHz] δ: 3.60 (2H, dd, *J* = 5.9, 11.6 Hz, H-1, 6), 3.70 (2H, m, H-2, 5), 3.73 (2H, t, *J* = 8.8 Hz, H-3, 4), 3.81 (2H, br d, *J* = 11.6 Hz, H-1, 6); ^{13}C-NMR (CD_3OD, 100 MHz) δ: 65.98 (C-1, 6), 72.01 (C-3, 4), 73.57 (C-2, 5); Acetate – ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 2.03 (6H, s, 2 × $OCOCH_3$), 2.05 (6H, s, 2 × $OCOCH_3$), 2.07 (6H, s, 2 × $OCOCH_3$), 4.04 (2H, dd, *J* = 5.0, 12.5 Hz, H-1a, 6a), 4.19 (2H, dd, *J* = 2.6, 12.5 Hz, H-1b, 6b), 5.05 (2H, m, H-2, 5), 5.43 (2H, br d, *J* = 9.0 Hz, H-3, 4); FABMS *m/z* 183 $[M + H]^+$.

Table 1. ^{13}C-NMR spectral data of iridoids (**2**, **3**, **5** – **7**, and **8**)

Carbon No.	**2**[1)]	**3**[2)]	**5**[2)]	**6**[2)]	**7**[2)]	**8**[2)]
1	94.7	97.8	97.7	98.3	98.1	98.6
3	152.4	153.2	154.2	154.5	153.4	154.5
4	109.8	111.7	110.5	110.8	111.9	105.3
5	27.3	29.4	33.6	29.9	30.1	22.8
6	42.6	33.2	29.8	36.2	38.7	30.2
7	217.5	104.5	157.0	175.6	181.5	103.3
8	43.5	135.8	135.3	135.3	135.4	133.4
9	45.6	45.3	45.4	46.2	45.8	43.6
10	13.2	119.8	120.4	121.2	120.5	121.1
11	167.0	169.2	169.0	169.6	169.7	167.5
$COO\underline{C}H_3$	50.9	51.7	52.0	52.4 52.8	52.1	
OCH_3		52.6 53.9				57.0
1'	101.0	100.1	100.2	100.8	100.3	100.3
2'	74.6	74.6	74.6	75.4	75.1	74.6
3'	78.4	78.0	77.9	78.8	78.3	78.0
4'	71.1	71.5	71.5	72.4	72.1	71.5
5'	78.9	78.4	78.0	79.2	78.9	78.4
6'	62.4	62.7	62.8	63.6	63.3	62.7
1"			97.4			
3"			152.2			
4"			109.3			
5"			31.0			
6"			143.2			
7"			197.0			
8"			135.5			
9"			46.4			
10"			119.4			
11"			169.1			
$COO\underline{C}H_3$			51.7			
1'''			99.8			
2'''			74.7			
3'''			77.9			
4'''			71.5			
5'''			78.2			
6'''			62.6			

[1)]pyridine-d_5; [2)]CD_3OD

Sucrose (10) – Amorphous white powder. $[\alpha]_D^{27}$ = +73.3° (*c* 0.84, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ: 3.31 (1H, t, *J* = 9.4 Hz, H-4'), 3.40 (1H, dd, *J* = 3.8, 10.0 Hz,

H-2'), 3.51 (2H, s, H-1), 3.60 (1H, t, J = 9.8 Hz, H-3'), 3.66 (2H, br s, H-6'), 3.67 (2H, br s, H-6), 3.69 – 3.75 (2H, m, H-5, 5'), 3.89 (1H, t, J = 8.5 Hz, H-4), 4.60 (1H, d, J = 8.8 Hz, H-3), 5.25 (1H, d, J = 3.7 Hz, H-1'); ^{13}C-NMR (D_2O, 75.5 MHz) δ: 62.4 (C-1), 104.6 (C-2), 77.4 (C-3), 75.0 (C-4), 82.3 (C-5), 63.6 (C-6), 93.1 (C-1'), 72.0 (C-2'), 73.3 (C-3'), 70.2 (C-4'), 73.5 (C-5'), 61.1 (C-6').

Results and Discussion

The flower buds of *L. japonica* were crushed, extract with 70% EtOH, and successively partitioned with H_2O and hexane, CH_2Cl_2, EtOAc, and then BuOH. The polar fractions of the EtOAc and BuOH soluble extracts were subjected to sequential column chromatography over silica gel, Sephadex LH-20, and RP-18 gel to afford ten components. The well-known iridoid derivatives from *Lonicera* spp. such as secologanin dimethyl acetal (**3**) and epivogeloside (**8**) (Kawai *et al*., 1988; Mehrotra *et al*., 1988; Tomassini *et al*., 1995) as well as D-mannitol (**9**), and sucrose (**10**) (Kim *et al*., 2006) were identified based on detailed NMR and MS analyses along with direct comparison with authentic samples.

Compound **1** was obtained as amorphous white powder and a series of pseudomolecular ion peaks were observed at *m/z* 838, 852, 866, and 880. The NMR data of **1** indicated the presence of a sugar (δ_H 4.94, 1H, d, J = 7.7 Hz, anomeric H; δ_C 105.5), an amide linkage (δ_H 8.60, 1H, d, J = 9.2 Hz, N-H; δ_C 175.6) and two long chain aliphatic moieties, suggesting a glycosphingolipid nature. The structure was characterized by comparison with the ^{13}C-NMR spectral data of glucocerebroside (Kang *et al*., 1999). Compound **1** showed a characteristic ^{13}C-NMR signal due to C-1 ~ C-4, C-1', C-2' and C-1" of a 1-*O*-β-glucopyranoside of a phytosphingosine-type ceramide possessing a 2-hydroxy fatty acid. The chemical shift of the H-2 at δ 5.31 and the carbon chemical shifts at δ 70.4 (C-1), 51.6 (C-2), 75.8 (C-3), 72.3 (C-4), 175.6 (C-1'), and 72.3 (C-2') were virtually identical with those of the reported data of other (2*S*,3*S*,4*R*)-phytosphingosine moieties (Kang *et al*., 1999; Kang *et al*., 2001). The positive FABMS spectrum of **1** showed a series of molecular ion $[M + Na]^+$ peaks at *m/z* 838, 852, 866, and 880 and fragment ion at *m/z* 500 $[\text{glucosyl-long chain base} + Na]^+$. A peak at *m/z* 682 $[(M + Na) - 162]^+$ and 664 $[(M + Na) - 179]^+$ indicated the loss of a hexose units. Methanolysis of **1** yield methyl glucoside, a mixture of fatty acid methyl ester and a long chain base. The fatty acid methyl esters were identified as methyl 2-hydroxybehenate, methyl 2-hydroxytricosanoate, methyl 2-hydroxylignocerate, and methyl 2-hydroxypentacosanoate by means of GC/MS analysis. The presence of a 2*S*,3*S*,4*R*,8*E/Z*-2-amino-1,3,4-trihydroxyoctadeca-8-ene long chain base was confirmed by the direct comparison with an authentic sample obtained from *Phytolacca americana* (Kang *et al*., 2001). Therefore, compound **1** was identified as 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E/Z*)-2-[(2*R*)-2-hydroxy(docosanoyl, tricosanoyl, tetracosanoyl, pentacosanoyl) amino]-8-octadecene-1,3,4-triol (Kim *et al*., 2008). Compound **2** exhibited a strong hydroxyl, 5-membered ring C=O, along with glycosidic C-O absorptions in the IR spectrum, and a pseudomolecular ion peak at *m/z* 411 $[M + Na]^+$ in positive FABMS. The NMR spectra were very close to those of loganin (Kim *et al*., 2009). However, the oxygenated methine carbon signal for C-7 was replaced by signal at δ 217.5 indicating the presence of a ketone group at this position. Therefore, compound **2** was established as 7-dehydrologanin (7-ketologanin) (Anikina *et al*., 1989; Gross *et al*., 1986). Compounds **6** and **7** were shown to be secologanin type secoiridoid structures by their characteristic ^{1}H-NMR data. Diagnostic features in the ^{1}H-NMR spectrum of **7** revealed an olefinic proton signal for H-3 [δ_H 7.40 (1H, d, J = 1.6 Hz)], a signal for a vinyl group [δ_H 5.18 (1H, dd, J = 1.6, 10.5 Hz, H-10a), δ_H 5.25 (1H, d, J = 17.2 Hz, H-10b), and δ_H 5.66 (1H, dt, J = 9.9, 17.1 Hz, H-8)], an acetal proton signal for H-1 [δ_H 5.47 (1H, d, J = 3.9 Hz)], and an anomeric proton signal for β-glucopyranosyl unit [δ_H 4.63 (1H, d, J = 7.9 Hz)], respectively. A comparison of ^{1}H- and ^{13}C-NMR spectral data of **6** with those of **7** indicated that the two compounds are very similar except for the presence of a signal for the carbomethoxyl group attached at C-7 as shown in Table 1. Based on the above data, the structures of **6** and **7** were dimethyl secologanoside (Mehrotra *et al*., 1988) and secoxyloganin, respectively (Calis & Sticher, 1984). The NMR spectra of **5** showed two characteristic sets of signals for vinyl, olefinic, glucosyl and carbomethoxyl, indicating that **5** was a dimer of secoiridoid glucoside. The positive FABMS spectrum gave pseudomolecular ions at *m/z* 781 $[M + Na]^+$ and 759 $[M + H]^+$ together with ions at *m/z* 619 $[(M + Na) - 162]^+$, 597 $[(M + H) - 162]^+$, and 435 $[(M + H) - (2 \times 162)]^+$. When ^{13}C-NMR spectral data of **5** were compared with those of **6** and **7**, significant differences were observed in the signals around C-6 (6") and C-7 (7") as indicated in Table 1. In the ^{1}H-NMR spectrum of **5**, signal of H-5" appeared at δ 4.04, which was correlated to C-7, 6" and C-7", while an olefinic signal of H-7 was observed at δ 6.71, which was correlated to C-5, 5" and C-7" in its HMBC spectrum.

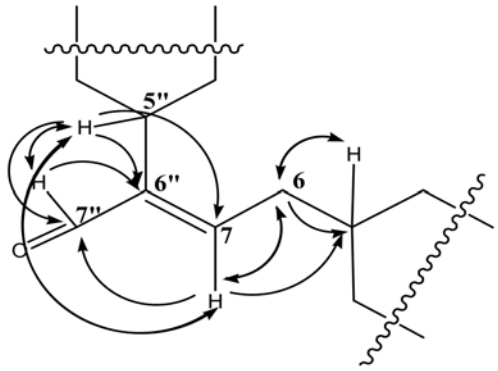

Fig. 1. ^{1}H-^{1}H COSY (↔) and HMBC (→) correlations for two secoiridoid positions (C-5 ~ C-7 and C-5" ~ C-7").

Therefore, two sets of secoiridoid glucosides were connected as indicated in Fig. 1. The stereochemistry of the C-7, 6" double bond in **5** was determined *E* configuration due to the presence of the deshielded aldehyde carbon signal at δ 197.0. From the above data, the structure of **5** was thus identified as (*E*)-aldosecologanin (centauroside) (Machida *et al*., 2002). Compound **4** was obtained as amorphous white powder. In the UV spectrum, **4** showed absorption band at 258 (3.50) nm, and showed absorptions at 1715 (CO), 1650 (CONH), 1451, 1418, 1233 (C-N), 821 cm^{-1} in the IR spectrum. The EIMS spectrum of **4** showed a molecular ion at *m/z* 112 as a base peak. In the ^{1}H-NMR spectrum of **4**, doublets at δ 7.50 (*J* = 7.5 Hz) and 5.80 (*J* = 7.5 Hz) assigned H-6 and H-5 of pyrimidine, respectively. Its ^{13}C-NMR spectrum of **4** showed two C-O signals at δ 165.7 and δ 153.2. Accordingly, the structure of **4** was elucidated as uracil (Lee *et al*., 2002).

A number of chemical constituents with diverse structures including six iridoid glycosides [7-dehydrologanin (7-ketologanin), secologanin dimethyl acetal, (*E*)-aldosecologanin (centauroside), dimethyl secologanoside, secoxyloganin, and epivogeloside], along with 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E/Z*)-2-[(2*R*)-2-hydroxy (docosanoyl, tricosanoyl, tetracosanoyl, pentacosanoyl)amino]-8-octadecene-1,3,4-triol, uracil, D-mannitol, and sucrose were isolated from polar extract of the flower buds of *L. japonica*.

This is the first report of 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E/Z*)-2-[(2*R*)-2-hydroxy(docosanoyl, tricosanoyl, tetracosanoyl, pentacosanoyl)amino]-8-octadecene-1,3,4-triol (**1**), 7-dehydrologanin (7-ketologanin, **2**), uracil (**4**), and sucrose (**10**) being isolated for the first time from the flower buds of *L. japonica*.

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