Loniceroside C, an Antiinflammatory Saponin from *Lonicera japonica*

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A new triterpenoid saponin, loniceroside C was isolated from the aerial parts of *Lonicera japonica*. Its structure was established to be 3-*O*-β-D-glucopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl (1→2)-[β-D-xylopyranosyl(1→6)]-β-D-glucopyranosyl ester by spectroscopic techniques and chemical transformations. Loniceroside C showed *in vivo* antiinflammatory activity against mouse ear edema provoked by croton oil.

Key words *Lonicera japonica*; Caprifoliaceae; triterpenoid saponin; loniceroside C; antiinflammatory activity

Lonicera japonica (THUNB.) (Caprifoliaceae) is a twining shrub that has been used as an antidote and to treat urinary disorders, fever, and headache.[1)] It has been known as an antiinflammatory agent in Korea from ancient times and is used widely for treating upper respiratory tract infections, diabetes mellitus, and rheumatoid arthritis.[2)] In a previous paper, we elucidated the structures of flavonoid derivatives[3)] as well as two new hederagenin glycosides, lonicerosides A and B.[4)] Furthermore, we reported that loniceroside A showed *in vivo* antiinflammatory activity in a croton-oil induced ear edema model and antiarthritic activity.[5,6)] In our continuing research on *L. japonica*, we isolated a new triterpenoid saponin, loniceroside C (**1**). This paper describes the structure elucidation and antiinflammatory activity of this compound.

Repeated silica gel and RP-18 chromatography of the BuOH fraction afforded a saponin (**1**). Compound **1** was positive in the Liebermann–Burchard and Molisch tests for saponins and its molecular formula was found to be $C_{53}H_{86}O_{22}$ by high-resolution FAB-MS [$(M+Na)^+$ at *m/z* 1097.5508, calcd., 1097.5509]. Acid hydrolysis of **1** yielded xylose, rhamnose, glucose, and an aglycone, hederagenin, identified by direct comparison with an authentic sample. The ^{1}H-NMR spectra of compound **1** indicated the presence of four anomeric proton signals at δ 4.84 (1H, d, J=7.4 Hz), 5.06 (1H, d, J=7.8 Hz), 6.09 (1H, d, J=8.1 Hz), and 6.49 (1H, s), which were in agreement with the ^{13}C-NMR signals for anomeric carbons at δ 105.7, 105.9, 94.8, and 101.6 by ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) and ^{13}C–^{1}H shift correlation spectroscopy (^{13}C–^{1}H COSY) (Table 1). In the ^{13}C-NMR spectra, two signals at δ 94.8 and 82.5 due to C-1 of inner glucose attached to C-28 of the hederagenin and C-3 of the hederagenin moiety, respectively, strongly indicated that compound **1** is a bisdesmosidic glycoside with an ester function at C-28. Alkaline hydrolysis of compound **1** gave a prosapogenin (compound **2**) which was identified as hederagenin 3-*O*-β-D-glucopyranoside by comparison of its NMR data with those previously reported for hederoside B.[7)] The results showed that the acyl moiety at C-28 is comprised of three sugars, *i.e.*, glucose, rhamnose, and xylose. The sugar sequence of the acyl moiety was determined by two-dimensional (2D) NMR analysis. In the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) spectrum, the long-range correlation between the H-1 of inner glucose (δ 6.09) and C-28 of aglycone (δ 176.6), H-1 of terminal rhamnose (δ 6.49) and C-2 of glucose (δ 75.5), and H-1 of terminal xylose (δ 4.84) and C-6 of glucose (δ 69.0) clearly demonstrated the presence of the 28-*O*-rhamnopyranosyl (1→2)-[xylopyranosyl (1→6)]glucopyranosyl moiety in compound **1** (Fig. 1). Furthermore, the ^{13}C-NMR signals of the three sugars were identical to those of the C-28 acyl moiety in lonicerosides A and B. Accordingly, the structure of compound **1** was determined to be 3-*O*-β-D-glucopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl (1→2)-[β-D-xylopyranosyl (1→6)]-β-D-glucopyranosyl ester.

Table 1. ^{13}C-NMR Spectral Data for Compound **1** in Pyridine-d_5[a)]

Carbon no.	**1**	Carbon no.	**1**	Carbon no.	**1**
1	38.8	20	30.7	28-*O*-Sugar moieties	
2	25.8	21	34.0	Glc 1	94.8
3	82.5	22	33.0	2	75.5
4	43.4	23	64.9	3	79.6
5	47.8	24	13.6	4	71.2
6	18.3	25	16.3	5	78.0
7	32.4	26	17.6	6	69.0
8	40.0	27	25.9	Rha 1	101.6
9	48.2	28	176.6	(→^{2}Glc) 2	72.2
10	37.0	29	33.1	3	72.6
11	23.9	30	23.9	4	73.9
12	122.8	3-*O*-Sugar moiety		5	69.8
13	144.2	Glc 1	105.9	6	18.8
14	42.3	2	75.9	Xyl 1	105.7
15	28.7	3	78.7	(→^{6}Glc) 2	74.8
16	23.4	4	71.6	3	77.6
17	47.2	5	78.3	4	71.1
18	42.0	6	62.8	5	67.0
19	46.4				

a) Assignments based upon ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, and HMBC experiments.

The new compound, loniceroside C (**1**), possesses *in vivo* antiinflammatory activity against mouse ear edema provoked by croton oil (Table 2). It inhibited ear edema (15.0—31.0% inhibition at 50—200 mg/kg). Reference compound, prednisolone, showed potent inhibition (57.9% inhibition at 10 mg/kg), while aspirin weakly inhibited ear edema (15.0% inhibition at 100 mg/kg). It is significant to note that **1** at the dose of 100 mg/kg showed antiinflammatory activity with potency similar to that of loniceroside A, previously reported to be one of active components.[5)] Therefore, **1** also contributes to the *in vivo* antiinflammatory activity of *L. japonica*.

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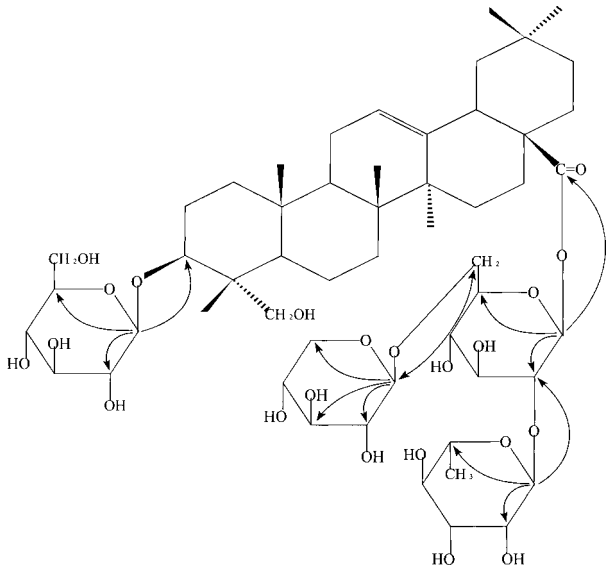

Fig. 1. Significant Long-Range Correlation of Loniceroside C (**1**) in the HMBC Spectrum

Table 2. Antiinflammatory Activity of Lonicerosides A and C (**1**) against Mouse Ear Edema Induced by Croton Oil

Group	Dose (mg/kg, *p.o.*)	Thickness increase (mm)	% Inhibition
Croton oil	—	0.126±0.011*a)*	—
Aspirin	100	0.107±0.019	15.0
Prednisolone	10	0.053±0.012*	57.9
Loniceroside A	100	0.088±0.018*	30.2
Loniceroside C (**1**)	50	0.107±0.019	15.0
	100	0.087±0.010*	31.0
	200	0.092±0.016	28.7

a) Arithmetic mean±S.D. (n=6), *: p<0.05, significantly different from the croton oil-treated group.

Experimental

General Procedures Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. The optical rotations were determined on a JASCO P-1020 polarimeter. The NMR spectra were measured on a Bruker AMX-500 instrument (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR), and the chemical shifts are referenced to tetramethylsilane (TMS). FAB-MS was obtained in a 3-nitrobenzyl alcohol matrix in positive-ion mode with a VG-VSEQ spectrometer. Gas chromatography (GC) analysis was performed with a Hewlett Packard 5890 Series II gas chromatograph equipped with a H_2 flame ionization detector. The conditions were HP-5 capillary column (30 m×0.32 mm×0.25 μm), column temperature, 200 °C; injector and detector temperature, 290 °C; and He flow rate, 1 ml/min. Column chromatography was carried out on Kieselgel 60 (Merck; 40—63 μm) and LiChroprep RP-18 (Merck; 40—63 μm). TLC was performed on precoated silica gel 60 F_{254} sheets (Merck), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Sugars were run on precoated cellulose plates (Merck) and detected using aniline phthalate.

Plant Material The aerial parts of *L. japonica* were collected in Kyungbug province, Korea, in the summer of 1998 and were authenticated by Dr. Hyung Joon Chi of the Natural Products Research Institute, Seoul National University. A voucher specimen is deposited in the Department of Food and Nutrition, Andong National University.

Animals Male ICR mice (specific pathogen free, 18—22 g) were purchased from Charles River, Japan. Animals had access to Purina lab. chow and water ad libitum and were maintained in our animal facility for at least 7 d prior to experiments at 21±1 °C, 40—60% relative humidity, and a 12 h/12 h (light/dark) cycle.

Extraction and Isolation Dried aerial parts of *L. japonica* (5 kg) were extracted with MeOH. The MeOH extract was evaporated *in vacuo* to give a residue (1.08 kg) that was suspended in H_2O and partitioned successively with hexane (41 g), $CHCl_3$ (5 g), EtOAc (16 g), and then with BuOH (45 g). The BuOH-soluble fraction (45 g) was subjected to column chromatography (CC) over silica gel with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.5, lower layer, and then 52 : 28 : 8, lower layer) to give nine subfractions. The eighth sub-fraction was further subjected to repeated CC over silica gel using EtOAc saturated with H_2O–MeOH (0—7%, gradient) and RP-18 with MeOH–H_2O (6 : 4) to obtain **1** (350 mg).

Loniceroside C (**1**) was obtained as amorphous powder from MeOH. mp 227—230 °C. $[\alpha]_D^{27}$ −26.7° (c=0.12, pyridine). IR (KBr) cm^{-1}: 3410 (OH), 2922 (C–H), 1741 (C=O), 1078 (glycosidic C–O). Positive-ion FAB-MS m/z: 1097.5508 $[M+Na]^+$ (Calcd for $C_{53}H_{86}O_{22}Na$: 1097.5509) ^{1}H-NMR (pyridine-d_5) δ: 0.79 (3H, s, H_3-29), 0.86 (3H, s, H_3-30), 0.90 (3H, s, H_3-24), 0.93 (3H, s, H_3-25), 1.06 (3H, s, H_3-26), 1.15 (3H, s, H_3-27), 1.71 (3H, d, J=6.2 Hz, H_3-6 of rhamnose), 4.84 (1H, d, J=7.4 Hz, H-1 of xylose), 5.06 (1H, d, J=7.8 Hz, H-1 of glucose attached to C-3 of aglycone), 5.37 (1H, br s, H-12), 6.09 (1H, d, J=8.1 Hz, H-1 of glucose attached to C-28 of aglycone), 6.49 (1H, s, H-1 of rhamnose). ^{13}C-NMR: Table 1.

Acid Hydrolysis of Compound 1 Compound **1** (20 mg) was refluxed with 4% H_2SO_4 (10 ml) in MeOH for 1 h. The reaction mixture was then concentrated under reduced pressure to remove MeOH, diluted with H_2O, and filtered. The precipitate was purified by recrystallization from MeOH to afford an aglycone, hederagenin (6 mg), as needles, which was identified by direct comparison with an authentic sample. The filtrate was adjusted to pH 7 with $BaCO_3$ and filtered. The filtrate was concentrated and examined by cellulose TLC (pyridine–EtOAc–HOAc–H_2O=36 : 36 : 7 : 21). Xylose (*Rf* 0.42), rhamnose (*Rf* 0.49), and glucose (*Rf* 0.28) were identified by comparison with authentic samples.

Alkaline Hydrolysis of Compound 1 Compound **1** (100 mg) in 3% KOH (in MeOH) was refluxed for 30 min. The reaction mixture was neutralized with 0.05 M H_2SO_4 and then extracted with BuOH. The BuOH layer was subjected to CC over silica gel to give the prosapogenin **2** (30 mg). Compound **2** was recrystallized from MeOH as an amorphous powder, mp 245—247 °C; $[\alpha]_D^{27}$ +44.5° (c=0.1, pyridine). ^{13}C-NMR (pyridine-d_5) δ 82.1 (C-3), 64.3 (C-23), 180.1 (C-28), 105.9 (C-1′), 76.1 (C-2′), 78.8 (C-3′), 71.9 (C-4′), 78.3 (C-5′), 62.8 (C-6′). Compound **2** was identified as hederagenin 3-*O*-β-D-glucopyranoside by comparison of its NMR data with those previously reported for hederoside B.[7]

Determination of the Absolute Configuration of Sugars of Compound 1 A sample of **1** (10 mg) was hydrolyzed with 1 M HCl (H_2O–dioxane, 1 : 1; 5 ml) at 80 °C for 3 h. The reaction mixture was neutralized with a small column of Amberlite IRA67 (OH^- form), and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in pyridine (0.1 ml), and then the solution was added to a pyridine solution (0.1 ml) of L-cysteine methyl ester hydrochloride (2 mg) and warmed at 60 °C for 1 h. The solvent was evaporated under a N_2 stream and dried *in vacuo*. Then trimethylsilylimidazole (0.1 ml) was added, and the mixture was heated at 60 °C for 1 h. After the addition of hexane and water (1 ml each), the hexane layer was analyzed by GC. The retention times of the peaks were 18.38 (D-xylose), 23.73 (L-rhamnose), and 37.87 min (D-glucose), respectively.

***In Vivo* Antiinflammatory Activity** To measure antiinflammatory (antiedema) activity *in vivo*, the croton oil-induced ear edema assay was carried out according to the modified procedure of Kim *et al.*[8] based on the original procedure of Tonneli *et al.*[9] In brief, the test compounds suspended in 0.5% carboxymethylcellulose were administered orally. One hour later, 2.5% croton oil (Sigma Chemical) dissolved in acetone (25 μl/ear) was applied topically to the inner and outer surfaces of mouse ears. After 5 h, ear thickness was measured using a spring-loaded dial thickness guage (Lux Scientific Instruments, U.S.A.). The increase in thickness compared with that before croton oil application was regarded as edematous inflammation. The statistical significance of the difference in ear thickness was evaluated using one-way analysis of variance (ANOVA).

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