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Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new triterpenoid saponins from the flowers and buds of *Lonicera japonica*

Li-Mei Lin[a]; Xiao-Guang Zhang[a]; Jing-Jing Zhu[a]; Hui-Min Gao[a]; Zhi-Min Wang[a]; Wei-Hao Wang[a]
[a] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

To cite this Article Lin, Li-Mei , Zhang, Xiao-Guang , Zhu, Jing-Jing , Gao, Hui-Min , Wang, Zhi-Min and Wang, Wei-Hao(2008) 'Two new triterpenoid saponins from the flowers and buds of *Lonicera japonica*', Journal of Asian Natural Products Research, 10: 10, 925 — 929

To link to this Article: DOI: 10.1080/10286020802217366

URL: http://dx.doi.org/10.1080/10286020802217366

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Journal of Asian Natural Products Research
Vol. 10, No. 10, October 2008, 925–929

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Two new triterpenoid saponins from the flowers and buds of *Lonicera japonica*

Li-Mei Lin, Xiao-Guang Zhang, Jing-Jing Zhu, Hui-Min Gao, Zhi-Min Wang* and Wei-Hao Wang

Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

(*Received 21 November 2007; final version received 12 May 2008*)

Two new triterpenoid saponins, loniceroside D (**1**) and loniceroside E (**2**), were isolated from the dry flowers and buds of *Lonicera japonica* Thunb., along with seven known compounds, chlorogenic acid (**3**), sweroside (**4**), vogeloside (**5**), *epi*-vogeloside (**6**), loniceroside A (**7**), loniceroside B (**8**), and loniceroside C (**9**). Their structures were elucidated on the basis of spectroscopic data, physicochemical properties, and acid hydrolysis.

Keywords: *Lonicera japonica* Thunb.; triterpenoid saponin; loniceroside D; loniceroside E

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1. Introduction

The flowers and buds of *Lonicera japonica* has been well known as an antiviral, anti-inflammatory, and antibacterial agent in traditional Chinese medicine and widely used in the treatment of various diseases, including upper respiratory tract infections, fever, sores, and swelling. Numerous compounds such as alkaloids, cerebrosides, flavonoids, iridoids, polyphenols, and triterpenoid saponins have been reported from various parts of this plant [1–3]. As a part of this study to investigate the active ingredients of herb couple consisting of *Flos Lonicerae japonicae* and *Fructus Forsythiae*, two new triterpenoid saponins (Figure 1), loniceroside D (**1**) and loniceroside E (**2**), along with seven known compounds, chlorogenic acid (**3**), sweroside (**4**), vogeloside (**5**), *epi*-vogeloside (**6**), and lonicerosides A (**7**), B (**8**), and C (**9**), were isolated from the aqueous extract of the dry flowers and buds of *L. japonica*. Their structures were elucidated on the basis of spectroscopic data, physico-chemical properties, and acid hydrolysis.

2. Results and discussion

Compound **1** was obtained as white powder and positive in Liebermann–Burchard and Molish tests for saponins. It was determined to have the molecular formula $C_{53}H_{86}O_{23}$ from negative HR-ESI-MS at *m/z* 544.2721 $[M-2H]^{2-}$ and positive ESI-MS at *m/z* 1113.8 $[M+Na]^{+}$. ^{1}H NMR spectrum showed four anomeric protons at δ 6.12 (1H, d, $J = 8.0$ Hz), 5.63 (1H, d, $J = 8.0$ Hz), 5.11 (1H, d, $J = 7.6$ Hz), and 4.88 (1H, d, $J = 7.6$ Hz); an olefinic proton at δ 5.39 (1H, br s), as well as six tertiary methyl resonances at δ 1.15, 1.02, 0.89, 0.89, 0.85, and 0.82. ^{13}C NMR spectrum exhibited four anomeric carbons at δ 105.8, 105.7, 104.7, and 93.5, and a pair of olefinic carbon at δ 122.6 and 144.4, along with an ester carbonyl carbon at δ 176.5. In addition, acid hydrolysis of **1** with 2 M trifluoroacetic acid (TFA) gave heder-agenin as aglycone, together with glucose and xylose, which were identified by comparison of their NMR spectral data with those previously reported in the literature [4–7]

*Corresponding author. Email: zhmw123@263.net

ISSN 1028-6020 print/ISSN 1477-2213 online
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DOI: 10.1080/10286020802217366
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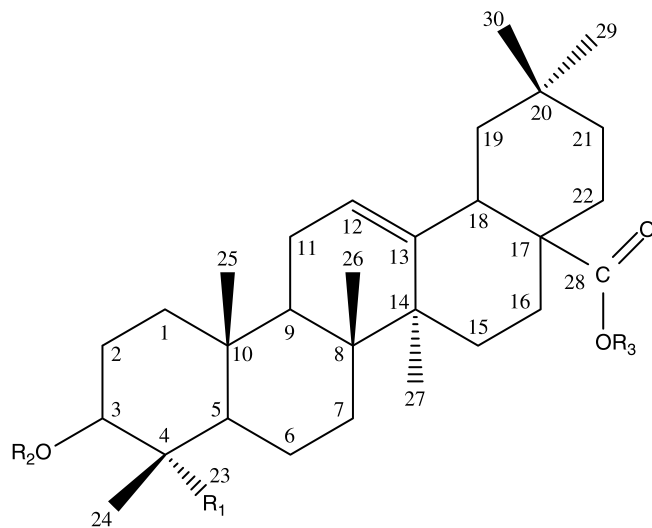

1 R_1=CH_2OH, R_2=glc, R_3=glc(1→2)[xyl(1→6)]glc

2 R_1=CH_3, R_2=glc, R_3=rham(1→2)[xyl(1→6)]glc

7 R_1=CH_2OH, R_2=ara, R_3=rham(1→2)[xyl(1→6)]glc

8 R_1=CH_2OH, R_2=rha (1→2) ara, R_3=rham(1→2)[xyl(1→6)]glc

9 R_1=CH_2OH, R_2=glc, R_3=rham(1→2)[xyl(1→6)]glc

Figure 1. Structures of triterpenoid saponins **1**, **2**, and **7**–**9**.

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and co-chromatographic examinations on thin layer plates with authentic samples. From the above spectroscopic and chemical information, compound **1** was deduced to be a bisdesmosidic triterpene saponin with four sugar units, disubstituted at the positions C-3 and C-28. ^{13}C NMR spectral data of **1** being compared with those of the reported loniceroside C (**9**); compound **1** was in accordance with **9**, except for a different sugar unit in the ester chain attached to position C-28 of the aglycone (Table 1). The rhamnopyranosyl unit in **9** was replaced by a xylopyranosyl unit in **1**. Furthermore, the exact sugar sequence and its linkage position to the aglycone were confirmed by detailed analysis of the 2D-NMR spectra. The key HMBC correlations from H-1 of glucopyranosyl unit at δ 5.11 to C-3 of aglycone at δ 82.1, H-1 of xylopyranosyl unit at δ 4.88 to C-6$'$ of inner glucopyranosyl unit at δ 68.8, H-1$''$ of another glucopyranosyl unit at δ 5.63 to C-2$'$ of inner glucopyranosyl unit at δ 75.9, and H-1$'$ of inner glucopyranosyl unit at δ 6.12 to C-28 of aglycone at δ 176.5 clearly indicated the presence of 3-*O*-glucopyranosyl moiety and 28-*O*-glucopyranosyl(1 → 2)-[xylopyranosyl(1 → 6)]-glucopyranosyl moiety in compound **1**. Accordingly, compound **1** was identified as 3-*O*-β-D-glucopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester, named loniceroside D.

Compound **2** was isolated as white powder and positive in Liebermann–Burchard and Molisch tests for saponins. Its positive ESI-MS and negative HR-ESI-MS exhibited quasimolecular ions at *m/z* 1081 $[M + Na]^+$, 1097 $[M + K]^+$, and 528.2747 $[M - 2H]^{2-}$, respectively, which indicated the molecular formula of compound **2** to be $C_{53}H_{86}O_{21}$. ^{1}H NMR spectrum suggested four anomeric protons at δ 6.56 (1H, br s), 6.12 (1H, d, $J = 8.0$ Hz), 4.92 (1H, d, $J = 7.6$ Hz), and 4.88 (1H, d, $J = 7.2$ Hz); an olefinic proton at δ 5.42 (1H, br s); and seven tertiary

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Table 1. ^{1}H and ^{13}C NMR spectral data of **1** (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, C_5D_5N).

Carbon no.	δ_C	Carbon no.	δ_C	Hydrogen no.	δ_H
1	38.7	C-3 glc-1	105.8	3	4.27
2	25.8	2	75.9	12	5.39 (1H, brs)
3	82.1	3	78.3	23	4.28
4	43.4	4	70.4	24	0.89 (3 h, s)
5	47.6	5	78.7	25	0.89 (3 h, s)
6	18.2	6	63.8	26	1.02 (3 h, s)
7	32.3	C-28 glc-1′	93.5	27	1.18 (3 h, s)
8	40.0	2′	78.5	29	0.90 (3 h, s)
9	48.2	3′	78.3	30	0.83 (3 h, s)
10	36.9	4′	72.8	C-3 glc-1	5.11 (1H, d, $J = 7.6$ Hz)
11	23.8	5′	77.7	2	4.06
12	122.6	6′	68.8	3	4.09
13	144.4	glc-1″	104.7	4	4.26
14	42.1	2″	75.9	5	4.37
15	29.1	3″	78.3	6	4.65, 4.37
16	23.0	4″	71.6	C-28 glc-1′	6.12 (1H, d, $J = 8.0$ Hz)
17	47.0	5″	78.8	2′	4.01
18	41.7	6″	62.8	3′	4.07
19	46.2	xyl-1	105.7	4′	4.10
20	30.7	2	74.8	5′	3.99
21	34.0	3	78.2	6′	4.63, 4.30
22	32.9	4	71.1	glc-1″	5.63 (1H, d, $J = 8.0$ Hz)
23	64.6	5	67.1	2″	4.06
24	13.6			3″	4.10
25	16.7			4″	4.21
26	17.5			5″	4.18
27	26.2			6″	4.50, 4.38
28	176.6			xyl-1	4.88 (1H, d, $J = 7.6$ Hz)
29	33.1			2	3.99
30	23.8			3	3.98
				4	4.19
				5	4.63

methyl signals at δ 1.26, 1.23, 1.07, 0.95, 0.91, and 0.86 ($\times 2$). The three-proton doublet at δ 1.76 (3H, d, $J = 6.4$ Hz) indicated the presence of a deoxyhexopyranosyl unit in compound **2**. Acid hydrolysis of **2** with 2 M TFA gave oleanolic acid as aglycone, together with glucose, rhamnose, and xylose. In ^{13}C NMR spectrum, C-3 and C-28 carbon signals were observed at δ 88.8 and 176.5, which further confirmed that compound **2** was a oleane bisdesmosidic glycoside, disubstituted at the positions C-3 and C-28. Comparison of NMR spectral data of **2** and **9** showed that compound **2** had the same sugar chains as **9**, but differed in the aglycone moiety (Table 2). The aglycones of compounds **2** and **9** were oleanolic acid and hederagenin, respectively. The HMBC correlations further completed the assignment of the sugar sequence and its linkage position. The HMBC correlations from H-1 of glucopyranosyl unit at δ 4.91 to C-3 of aglycone at δ 88.8, H-1 of xylopyranosyl unit at δ 4.87 to C-6′ of inner glucopyranosyl unit at δ 69.0, H-1 of rhamnopyranosyl unit at δ 6.56 to C-2′ of inner glucopyranosyl unit at δ 75.2, and H-1′ of inner glucopyranosyl unit at δ 6.12 to C-28 of aglycone at δ 176.5 manifested the presence of 3-*O*-glucopyranosyl moiety and 28-*O*-rhamnopyranosyl(1 → 2)-[xylopyranosyl(1 → 6)]-glucopyranosyl moiety in compound **2**. Thus, compound **2** was determined as 3-*O*-β-D-glucopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester, named loniceroside E.

Table 2. ^{1}H and ^{13}C NMR spectral data of **2** (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, C_5D_5N).

Carbon no.	δ_C	Carbon no.	δ_C	Hydrogen no.	δ_H
1	38.8	C-3 glc-1	106.9	3	3.65 (1H, dd, J = 4.0 Hz, 11.6 Hz)
2	26.6	2	75.8	12	5.42 (1H, brs)
3	88.8	3	78.8	23	1.23 (3 h, s)
4	39.5	4	71.8	24	0.95 (3 h, s)
5	55.9	5	78.3	25	0.91 (3 h, s)
6	18.5	6	63.0	26	1.07 (3 h, s)
7	32.3	C-28 glc-1′	94.7	27	1.26 (3 h, s)
8	39.9	2′	75.2	29	0.87 (3 h, s)
9	48.0	3′	79.7	30	0.87 (3 h, s)
10	37.0	4′	71.2	C-3 glc-1	4.92 (1H, d, J = 7.6 Hz)
11	23.8	5′	78.0	2	4.07
12	122.7	6′	69.0	3	4.24
13	144.2	rha-1	101.4	4	4.24
14	42.3	2	72.3	5	4.01
15	28.7	3	72.5	6	4.59, 4.40
16	23.4	4	73.8	C-28 glc-1′	6.12 (1H, d, J = 8.0 Hz)
17	47.2	5	69.8	2′	4.39
18	42.0	6	18.8	3′	4.26
19	46.4	xyl-1	105.6	4′	4.31
20	30.7	2	74.8	5′	4.11
21	34.1	3	77.6	6′	4.64, 4.29
22	33.2	4	71.0	rha-1	6.56 (1H, brs)
23	28.3	5	67.0	2	4.76
24	17.0			3	4.53
25	15.6			4	4.34
26	17.5			5	4.53
27	25.9			6	1.76 (3 h, d)
28	176.5			xyl-1	4.88 (1H, d, J = 7.2 Hz)
29	33.3			2	3.95
30	23.9			3	4.09
				4	4.15
				5	4.30

3. Experimental

3.1 General experimental procedures

^{1}H NMR, ^{13}C NMR and HMBC spectra were recorded on a Bruker AM-400 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, respectively) with TMS as an internal standard. ESI-MS were performed on mass spectrometer (Micromass Zabspec); HR-ESI-MS were recorded on Trace-MS. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Oceanic Chemical Industry, Qingdao, China), RP-C_{18} (40–75 μm, Fuji Silysia Chemical Ltd, Kasugai, Japan), macroporous resin HPD_{100} (Hebei Cangzhou Chemical Ltd, Cangzhou, China), polyamide (60–100 mesh, Roden Company of Shanghai, Shanghai, China), and Sephadex LH-20 (GE Health Bio-Sciences AB, Uppsala, Sweden). Glucose, xylose, and rhamnose of authentic samples were purchased from Sigma (St Louis, USA).

3.2 Plant material

The dry flowers and buds of *L. japonica* Thunb. were collected in Henan Province and identified by associate professor Xi-Rong He, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences. The voucher specimen (20060428-3) is deposited in this institute.

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3.3 Extraction, isolation, and identification

The dry flowers and buds of *L. japonica* (3.5 kg) were decocted thrice and the aqueous extract was filtered. The filtrate was chromatographed by macroporous resin HPD_{100} eluted with gradient solvent systems of H_2O–EtOH (100:0, 30:70, 60:40) to yield fractions A, B, and C. Fraction A was separated on polyamide and Sephadex LH-20 column chromatography to give compound **3** (50 mg); fractions B and C were subjected to vacuum liquid chromatography, and eluted with $CHCl_3$, EtOAC, and *n*-butanol, in turn, to obtain fractions B_1, B_2, and B_3, and fractions C_1, C_2, and C_3, respectively. Fraction B_2 was separated on silica gel column chromatography eluted with solvent system of $CHCl_3$–MeOH–H_2O (40:8:1) to yield compounds **4** (12 mg), **5** (18 mg), and **6** (192 mg); fraction C_3 was purified on silica gel column chromatography ($CHCl_3$–MeOH–H_2O 65:35:10); and RP-C_{18} column chromatography to afford compounds **1** (47 mg), **2** (56 mg), **7** (45 mg), **8** (72 mg), and **9** (86 mg).

*3.3.1 Loniceroside D (**1**)*

White powder. ^1H and ^{13}C NMR spectral data are listed in Table 1. HR-ESI-MS (*m/z*): 544.2721 $[M - 2H]^{2-}$ (calcd for $C_{53}H_{86}O_{23}/2$, 544.2707). ESI-MS (*m/z*): 1113.8 $[M + Na]^+$.

*3.3.2 Loniceroside E (**2**)*

White powder. ^1H and ^{13}C NMR spectral data are listed in Table 2. HR-ESI-MS (*m/z*): 528.2747 $[M - 2H]^{2-}$ (calcd for $C_{53}H_{85}O_{21}/2$, 528.2758). ESI-MS (*m/z*): 1081.8 $[M + Na]^+$, 1097.8 $[M + K]^+$.

3.4 Acid hydrolysis of saponins

Compounds **1** (15.1 mg) and **2** (17.3 mg) in 2.0 M TFA were sealed and placed in oven at 110°C for 6 h, respectively. The reaction mixture was extracted with EtOAc (10 ml × 2) when white flocculent precipitate appeared. The EtOAc extract and aqueous layer were condensed and analyzed by silica gel TLC and colorated by 1% vanillin–H_2SO_4, respectively. The EtOAc extract and the authentic sample of hederagenin or oleanolic acid had the same R_f values and colorful spots on TLC chromatogram. The sugar moiety was identified by the chemical shifts of ^1H and ^{13}C NMR spectra and comparison of the R_f value on TLC chromatogram with those of the authentic samples of glucose, rhamnose, and xylose [8].

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Acknowledgements

This project was supported by the National Natural Science Foundation of China (Grant No. 30672601). NMR spectra were measured by Institute of Materia Medica, Chinese Academy of Medical Sciences; Mass spectra were operated at the Center of Chemical Institution of Chinese Academy of Sciences and Academy of Military Medical sciences.

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