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New Triterpenoid Saponins from the Herb *Hylomecon japonica*

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Abstract: *Background:* *Hylomecon japonica*, a plant of the *Papaveraceae* family which is well-known for the alkaloids they produce, is a perennial plant widely distributed in the northeast, central and east regions of China. Although a variety of chemical constituents, including alkaloids, flavonoids, and megastigmoids, have been isolated from *H. japonica*, the investigation of saponins in *H. japonica* has not been reported until now. *Methods:* Various separation techniques, including polyporous resin column chromatography, silica gel column chromatography and semi-preparative HPLC were applied to the isolation of triterpenoid saponins, and chemical methods such as acid hydrolysis and spectroscopic methods including HRESIMS and NMR were applied to their structure elucidation, and the XTT reduction method was used to assay cytotoxicity. *Results:* Two new triterpenoid saponins, named hylomeconoside A (1) and B (2) which were identified as 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-quinovopyranoside (1) and 3-O-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl-gypsogenin-28-O-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (2), and two known triterpenoid saponins identified as dubiouside C (3) and lucyoside P (4) on the basis of spectroscopic and chemical evidence, were isolated from *H. japonica*. Compound 1 exhibited moderate cytotoxicity on MGC-803 and HL-60 cells, with IC₅₀ values of 43.8 and 32.4 μg·mL⁻¹, respectively. *Conclusions:* Compounds 1 and 2 are new saponins, and 1 is considered to be one of the antitumor principles in this plant. This is the first time that triterpenoid saponins have been isolated from plants of the *Papaveraceae* family.

Keywords: *Hylomecon japonica*; *Papaveraceae*; triterpenoid saponin; gypsogenin; quillaic acid

1. Introduction

Hylomecon japonica (Thunb.) Prantl et Kundig (*Papaveraceae*) is a perennial plant widely distributed in the northeast, central and east regions of China. Its roots have been used as a traditional Chinese medicine for the treatment of rheumatism and injuries [1]. Although a variety of chemical constituents, including alkaloids, flavonoids, and megastigmoids, were previously isolated from *H. japonica*, and pharmacological studies on its anti-inflammatory properties, antibacterial activities, and antitumor action have been reported [2–7], the investigation of saponins in *H. japonica* has not been reported until now. As part of our study on the plants of *Papaveraceae* family, we have been carrying out an

investigation of saponins in *H. japonica*. In this paper, we now report the isolation and characterization of two new triterpenoid saponins, named hylomeconoside A (**1**) and B (**2**), which were identified as 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl-gypsogenin-28-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-quinovopyranoside (**1**) and 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl-gypsogenin-28-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside (**2**), and two known triterpenoid saponins identified as dubioside C (**3**) [8], and lucyoside P (**4**) [9] (Figure 1) on the basis of spectroscopic and chemical evidence.

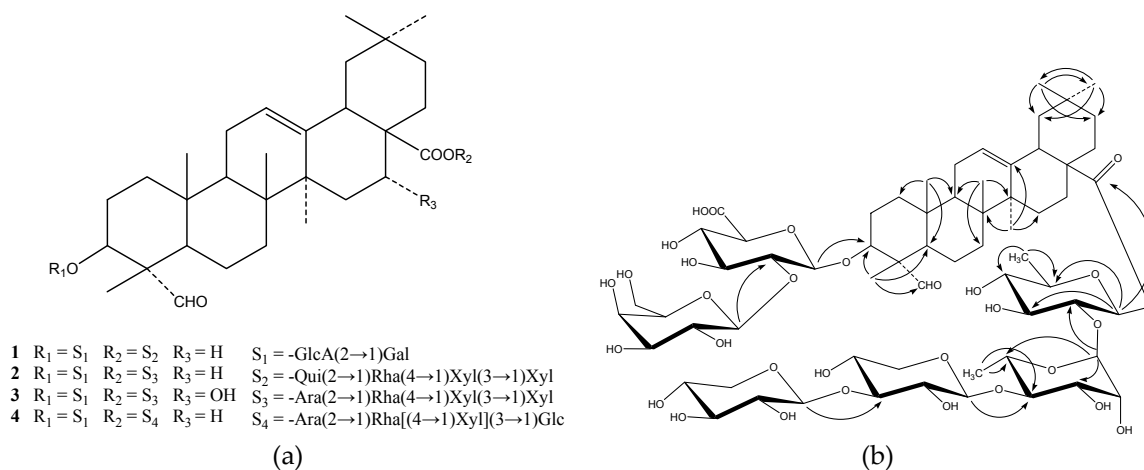


Figure 1. (a) Chemical structures of compounds 1–4; (b) Key HMBC correlations of compound 1 (arrows point from proton to carbon).

2. Results and Discussion

The crude saponins prepared from the 70% EtOH extract of *H. japonica* herbs through a D101 polyporous resin column were subjected to silica gel chromatographies and semi-preparative HPLC to yield two new triterpenoid saponins, named as hylomeconoside A (**1**) and B (**2**), and two known triterpenoid saponins identified as dubioside C (**3**) [8] and lucyoside P (**4**) [9] on the basis of spectroscopic and chemical evidence.

Hylomeconoside A (**1**) was obtained as a white amorphous solid. The molecular formula of **1** was determined as $C_{64}H_{100}O_{31}$ by high-resolution HRESI-MS which indicated a $[M + H]^+$ ion at m/z 1365.6318. Acid hydrolysis of **1** afforded an aglycone which was identified as gypsogenin on the basis of the 1H - and ^{13}C -NMR data [10]. The sugars obtained from the saponin hydrolysate were identified as D-xylose, L-rhamnose, D-quinovose, D-glucuronic acid and D-galactose based on GC analysis of their chiral derivatives. The 1H -NMR spectrum revealed signals due to six quaternary methyls at δ_H 0.68, 0.76, 0.79, 0.90, 1.14, 1.28, an olefinic proton at δ_H 5.30 (br. s), an aldehyde proton at δ_H 9.83 (s), and six anomeric protons at δ_H 6.34 (br s), 5.98 (d, $J = 8.0$ Hz), 5.11 (d, $J = 7.5$ Hz), 5.08 (d, $J = 7.4$ Hz), 4.94 (d, $J = 6.8$ Hz), and 4.72 (d, $J = 6.3$ Hz). The ^{13}C -NMR spectrum displayed signals due to six quaternary carbon at δ_C 30.9, 36.4, 40.4, 42.4, 47.3, and 55.2, an oxygen-bearing methine carbon at δ_C 83.5, a set of olefinic carbons at δ_C 122.7 and 144.2, an ester carbonyl carbon at δ_C 176.7, an aldehyde carbon at δ_C 210.2, and six anomeric carbons at δ_C 94.5, 101.4, 103.4, 105.9, 106.2, and 106.9. All the above spectral information suggested that the aglycone moiety of **1** was gypsogenin and the chemical shift of the carbonyl C-28 (δ_C 176.7) and the deshielded carbon C-3 (δ_C 83.5) of gypsogenin indicated that **1** was the 3,28-bisdesmoside of gypsogenin, having six monosaccharide units.

The six anomeric proton signals at δ_H 6.34 (br s), 5.98 (d, $J = 8.0$ Hz), 5.11 (d, $J = 7.5$ Hz), 5.08 (d, $J = 7.4$ Hz), 4.94 (d, $J = 6.8$ Hz), and 4.72 (d, $J = 6.3$ Hz) were correlated with anomeric carbon signals at δ_C 101.4, 94.5, 106.2, 105.9, 106.9, 103.4, respectively, and the analysis of 1H - 1H COSY, HMQC, HMBC, DEPT NMR and spin-spin couplings in 1H -NMR of **1** allowed the identification of

one α -L-rhamnopyranosyl (Rha), one β -D-quinovopyranosyl (Qui), one β -D-glucuronopyranosyl (GlcA), one β -D-galactopyranosyl (Gal) and two β -D-xylopyranosyl (Xyl) moieties. The β -anomeric configurations for D-xylopyranosyl, D-quinovopyranosyl, D-glucuronopyranosyl and D-galactopyranosyl moieties were determined by their large $^3J_{H1-H2}$ coupling constants of 6–8 Hz and the α -anomeric configuration for L-rhamnopyranosyl unit was determined by its small $^3J_{H1-H2}$ coupling constant.

One Xyl unit, Xyl^a, identified starting from anomeric signals at δ_H 5.08 (H-1 of Xyl^a) and δ_C 105.9 (C-1 of Xyl^a), was identified to be in the terminal position, as observed by its ^{13}C -NMR chemical shifts. Another Xyl unit, Xyl^b, identified starting from anomeric signals at δ_H 4.94 (H-1 of Xyl^b) and δ_C 106.9 (C-1 of Xyl^b), was substituted at the position of C-3 of Xyl^b based on the deshielding of C-3 of Xyl^b (δ_C 87.2), and the Xyl^a unit was attached to this position based on the long-range correlation observed in the HMBC experiment between signals at δ_H 5.08 (H-1 of Xyl^a) and δ_C 87.2 (C-3 of Xyl^b). The methyl doublet at δ_H 1.66 (3H, d, $J = 6.4$ Hz) and the typical broad singlet of anomeric proton at δ_H 6.34 (br s) were characteristic of Rha unit, and the deshielding of C-4 of Rha (δ_C 85.3) indicated a substitution at the position of C-4 of Rha, and the long-range correlation between signals at δ_H 4.94 (H-1 of Xyl^b) and δ_C 85.3 (C-4 of Rha) indicated that Xyl^b was attached to the position of C-4 of Rha. The Qui unit was identified starting from the deshielded anomeric proton at δ_H 5.98 ($J = 8.0$ Hz) and characterized by its methyl doublet at δ_H 1.42 (3H, d, $J = 6.0$ Hz). The deshielding of anomeric proton and the chemical shift of anomeric carbon at δ_C 94.5 suggested that Qui was attached by an ester linkage to the C-28 carboxylic group of the aglycone, which was further confirmed by the long-range correlation observed in the HMBC experiment between signals at δ_H 5.98 (H-1 of Qui) and δ_C 176.7 (C-28 of gypsogenin). Qui was substituted at the position of C-2 of Qui as observed by its deshielded H-2 of Qui (δ_H 4.23) and the long-range correlation observed in the HMBC experiment between signals of the Rha anomeric proton (δ_H 6.34) and C-2 of Qui (δ_C 76.6). Therefore, the sequencing of the ester chain was obtained by analysis of HMBC experiment which showed cross-peaks between H-1 of Rha (δ_H 6.34) and C-2 of Qui (δ_C 76.6), between H-1 of Xyl^b (inner xylose) (δ_H 4.94) and C-4 of Rha (δ_C 85.3), and between H-1 of Xyl^a (the terminal xylose) (δ_H 5.08) and C-3 of Xyl^b (δ_C 87.2), and thus the ester chain was a tetrasaccharide, β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-quinovopyranosyl unit.

The Gal unit, identified starting from anomeric signals at δ_H 5.11 (H-1 of Gal) and δ_C 106.2 (C-1 of Gal), was identified to be in terminal position, as observed by its ^{13}C -NMR chemical shifts. Starting from the anomeric proton at δ_H 4.72 (d, $J = 6.3$ Hz), a GlcA unit was identified with its carbonyl C-6 at δ_C 173.7. The deshielding of C-2 of GlcA (δ_C 82.1) indicated a substitution of glucuronic acid. Observation of long-range proton-carbon correlations in the HMBC spectrum between the anomeric proton of GlcA (δ_H 4.49) and C-3 of gypsogenin (δ_C 83.5) and between the anomeric proton of Gal (δ_H 5.11) and C-2 of GlcA (δ_C 82.1) indicated a disaccharide chain attached at C-3 of gypsogenin, β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl unit.

The complete assignment of the signals of **1** was based on DEPT ^{13}C -NMR and 2D NMR of 1H - 1H COSY, HMQC and HMBC. All the data of 1H -, ^{13}C -NMR and 2D-NMR of **1** see Table 1, and the key correlations in HMBC NMR and the structure of **1** see Figure 1. In conclusion, compound **1** was identified as 3-O- β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl-gypsogenin-28-O- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- β -D-quinovopyranoside.

Hylomeconoside B (**2**) was obtained as a white amorphous solid. The molecular formula of **2** was determined as $C_{63}H_{98}O_{31}$ by high-resolution HRESI-MS which showed a $[M + H]^+$ ion at m/z 1351.6151. Acid hydrolysis of **2** afforded an aglycone which was identified as gypsogenin on the basis of the 1H - and ^{13}C -NMR spectra [10]. The sugars obtained from the saponin hydrolysate were identified as L-arabinose, D-xylose, L-rhamnose, D-glucuronic acid and D-galactose based on GC analysis of their chiral derivatives. In comparative analysis of the ^{13}C -NMR data of **2** with those of **1** and **3**, it was found that the spectra data of the aglycone moiety of **2** are same as those of **1**, and the spectra data of the sugar moiety of **2** are consistent with those of **3** (Table 2), which suggested

that the aglycone of **2** is gypsogenin and the structure of the sugar moiety is same as those of **3**. By further analysis of spectral data with the similar method as above, **2** was identified as 3-*O*- β -D-galactopyranosyl-(1 \rightarrow 2)- β -D-glucuronopyranosyl-gypsogenin-28-*O*- β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-xylopyranosyl-(1 \rightarrow 4)- α -L-rhamnopyranosyl-(1 \rightarrow 2)- α -L-arabinopyranoside.

Table 1. The spectral data of $^1\text{H-NMR}$ (400 MHz), $^{13}\text{C-NMR}$ (100 MHz), and 2D-NMR of $^1\text{H-}^1\text{H COSY}$ and HMQC of compound **1** in $\text{C}_5\text{D}_5\text{N}$.

Aglycone Moiety				Sugar Moiety			
Position	δ_{H} (J in Hz)	$^1\text{H-}^1\text{H COSY}$	δ_{C} HMQC	Position	δ_{H} (J in Hz)	$^1\text{H-}^1\text{H COSY}$	δ_{C} HMQC
1 α	0.69 m	H-1 β , H-2 β	38.3	3- <i>O</i> -			
1 β	1.26 m	H-1 α		GlcA-1	4.72 d (6.3)	H-2	103.4
2 α	2.04 m	H-2 β , H-3	25.2	2	4.06 m	H-1, H-3	82.1
2 β	1.73 m	H-2 α , H-1 α , H-3		3	4.12 m	H-2, H-4	77.0
3	3.90 m	H-2 α , H-2 β	83.5	4	4.28 m		73.2
4			55.2	5	4.27 m		77.9
5	1.25 m	H-6 β	48.7	6			173.7
6 α	1.26 m	H-6 β	20.8	Gal-1	5.11 d (7.5)	H-2	106.2
6 β	1.01 m	H-6 α , H-7 α		2	4.42 m	H-1, H-3	74.4
7 α	1.91 m	H-6 β , H-7 β	32.8	3	3.99 m	H-2	75.0
7 β	1.65 m	H-7 α		4	4.45 m		70.3
8			40.4	5	3.98 m	H-6	77.0
9	1.52 m	H-11 α , H-11 β	48.0	6	4.40 m	H-5	62.2
10			36.4	28- <i>O</i> -			
11 α	1.71 m	H-9, H-11 β	23.8	Qui-1	5.98 d (8.0)	H-2	94.5
11 β	1.79 m	H-11 α , H-9		2	4.23 t (8.0)	H-1, H-3	76.6
12 β	5.30 br.s	H-11 α , H-11 β	122.7	3	4.05 t (8.4)	H-2, H-4	79.3
13			144.2	4	3.55 t (8.8)	H-3, H-5	76.9
14			42.4	5	3.71 m	H-4, H-6	73.9
15 α	1.33 m	H-15 β	28.6	6	1.42 d (6.0)	H-5	18.5
15 β	1.90 m	H-15 α		Rha-1	6.34 br. s	H-2	101.4
16 α	2.04 m	H-16 β	23.9	2	4.69 br. s	H-1, H-3	71.8
16 β	1.76 m	H-16 α		3	4.54 dd (9.2, 2.8)	H-2, H-4	72.7
17			47.3	4	4.23 dd (8.4, 9.2)	H-3, H-5	85.3
18	3.02 m	H-19 α , H-19 β	42.3	5	4.36 m	H-4, H-6	68.3
19 α	1.63 m	H-18, H-19 β	46.4	6	1.66 d (6.4)	H-5	18.7
19 β	1.09 m	H-19 α , H-18		Xyl ^b -1	4.94 d (6.8)	H-2	106.9
20			30.9	2	3.89 m	H-1, H-3	75.2
21 α	1.20 m		34.1	3	3.91 m	H-2, H-4	87.2
21 β	1.03 m			4	3.97 m	H-3, H-5 α	69.0
22 α	1.44 m		32.5	5 α	3.37 t (10.4)	H-4, H-5 β	67.1
22 β	1.42 m			5 β	4.10 m	H-4, H-5 α	
23	9.83 s		210.2	Xyl ^a -1	5.08 (d, 7.4)	H-2	105.9
24	1.28 s		11.1	2	3.93 (m)	H-1	75.5
25	0.68 s		15.9	3	4.02 (m)		78.2
26	0.90 s		17.6	4	4.03 (m)	H-5 α	71.0
27	1.14 s		26.1	5 α	3.54 t (9.6)	H-4, H-5 β	67.4
28			176.7	5 β	4.15 m	H-4, H-5 α	
29	0.76 s		33.3				
30	0.79 s		23.9				

Dubioside C (**3**), one of active constituents of *Thladianthae dubiae* roots, has been reported to possess analgesic and anti-inflammatory effects [11–13] and lucyoside P (**4**), one of active constituents of *Luffa cylindrica* fructus, has beneficial effects on intelligence [14], therefore we have only carried out an activity assay on the new saponins **1** and **2**. The cytotoxic activities of compounds **1** and **2** on MGC-803 (human gastric cancer), HL-60 (human promyelocytic leukemia), BEL-7404 (human hepatoma carcinoma), MCF-7 (human breast cancer), and SPC-A1 (lung adenocarcinoma) cells were assessed by the XTT (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) reduction method.

Table 2. The ^{13}C -NMR data of compound 2–4 in $\text{C}_5\text{D}_5\text{N}$.

C No.	2	3	4	C No.	2	3	4
1	38.2	38.3	38.3	C-3-O-sugars			
2	25.0	24.9	25.1	GlcA-1	103.3	103.0	103.1
3	83.5	83.3	84.7	2	82.4	82.3	80.0
4	55.2	55.2	55.9	3	77.2	77.2	76.6
5	48.4	48.5	48.3	4	73.3	73.3	72.4
6	20.6	20.6	20.8	5	77.9	77.9	77.3
7	32.8	32.9	32.8	6	173.7	173.7	176.4
8	40.2	40.4	40.3	Gal-1	106.4	106.3	104.4
9	47.9	47.1	48.0	2	74.6	74.6	73.1
10	36.3	36.4	36.3	3	75.0	75.0	74.3
11	23.3	23.9	23.4	4	69.9	70.3	69.8
12	122.8	122.6	123.0	5	77.2	77.2	77.3
13	144.3	144.6	144.4	6	62.2	62.3	62.2
14	42.2	42.2	42.4	C-28-O-sugars			
15	28.4	36.2	28.4	Ara-1	93.6	93.6	93.6
16	23.9	74.1	23.9	2	75.1	75.2	75.9
17	47.4	49.6	47.7	3	69.9	69.9	69.9
18	41.8	41.4	41.9	4	66.2	66.1	66.4
19	46.3	47.1	46.5	5	63.1	63.1	63.1
20	31.0	31.1	31.0	Rha-1	101.0	101.1	101.2
21	34.2	36.1	34.2	2	71.9	72.0	71.2
22	32.6	32.3	32.6	3	72.8	72.8	82.2
23	209.9	209.6	211.8	4	84.0	83.5	78.6
24	11.1	11.0	10.8	5	68.6	68.6	69.0
25	15.8	15.9	15.9	6	18.5	18.5	18.7
26	17.5	17.6	17.6	Xyl(inner)-1	106.6	106.3	
27	26.2	27.3	26.3	2	75.2	75.1	
28	176.4	176.0	177.6	3	87.1	87.2	
29	33.3	33.4	33.4	4	69.1	69.1	
30	23.8	24.9	23.9	5	67.0	67.0	
				Xyl(terminal)-1	106.4	106.1	104.6
				2	75.4	75.4	75.2
				3	78.3	78.3	76.6
				4	71.0	71.0	70.8
				5	67.4	67.4	66.5
				Glc(terminal)-1			105.4
				Glc-2			72.9
				Glc-3			77.5
				Glc-4			70.2
				Glc-5			77.8
				Glc-6			62.0

The results (Table 3) showed that compound 1 exhibited moderate cytotoxicity on MGC-803 and HL-60, with IC_{50} values of 43.8 and 32.4 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively. Therefore, compound 1 is considered to be one of the antitumor principles in this plant.

Table 3. The cytotoxicity of compounds **1** and **2** assayed by XTT assay (n = 3).

Comps.	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	Inhibitory Rate, Mean \pm SD (%)				
		MGC-803	HL-60	BEL-7402	MCF-7	SPC-A1
1	10	9.3 \pm 1.3 ¹	11.3 \pm 1.6	0	0	0
	20	18.5 \pm 1.1	19.7 \pm 1.5	0	0	0
	40	43.2 \pm 1.6	58.4 \pm 1.3	0	0	0
	80	75.0 \pm 2.3	91.7 \pm 2.1	0	0	25.1 \pm 1.2
	160	92.1 \pm 1.7	98.2 \pm 1.9	22.0 \pm 1.7	25.2 \pm 1.8	40.1 \pm 2.2
2	10	0	0	0	0	0
	20	0	0	0	0	0
	40	10.2 \pm 1.8	9.2 \pm 1.3	0	0	0
	80	18.4 \pm 1.6	20.3 \pm 1.2	0	0	0
	160	44.3 \pm 1.5	55.0 \pm 2.2	22.0 \pm 1.9	25.20 \pm 1.8	23.1 \pm 2.1

¹ Results are expressed as mean \pm standard deviation. The IC₅₀ values of compound **1** is 43.8 $\mu\text{g}\cdot\text{mL}^{-1}$ on MGC-803 and 32.4 $\mu\text{g}\cdot\text{mL}^{-1}$ on HL-60.

3. Materials and Methods

3.1. General Information

NMR spectra were recorded on an AV-400 spectrometer (Bruker Corporation, Faellanden, Switzerland). HRESI-MS were recorded on a Bruker microOTOF-Q II mass spectrometer (Bruker Corporation, Bremen, Germany). Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter (Horiba Ltd, Kyoto, Japan). HPLC was performed on a Shimadzu LC-10A system equipped with a SPD-10A detector (Shimadzu Corporation, Kyoto, Japan) and a Gemini 5 μm C18 110A column (250 mm \times 10.00 mm, 5 μm , Phenomenex, Torrance, CA, USA). GC was performed on an Agilent 7820A gas chromatograph with a quartz capillary column (30 mm \times 0.32 mm \times 0.25 μm , Agilent Technologies Inc., Santa Clara, CA, USA); detection, FID. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), D101 polyporous resin (Tianjin Pesticide Co., LTD., Resin Branch, Tianjin, China). TLC was performed on glass precoated silica gel GF₂₅₄ plates (Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), detection under UV light or by heating after spraying with 10% H₂SO₄ in 95% EtOH. Distilled water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). Acetonitrile of chromatographic grade for HPLC was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other chemicals and reagents of analytical grade were from Beijing Chemical Works (Beijing, China).

The bioactivities were measured on a DNM-9602 enzyme immunoassay spectrophotometer (Beijing, China). RPMI 1640 medium was purchased from HyClone (Logan, UT, USA), PBS from Gibco company (Carlsbad, CA, USA), fetal bovine serum (FBS) from Zhejiang Tianhang Biotechnology Co., Ltd. (Hangzhou, China), XTT (2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) and phenazine methosulphate from Sigma-Aldrich Shanghai Trading Co. Ltd. (Shanghai, China).

The *Hylomecon japonica* herb was collected in the Changchun District of Jilin Province, China. They were identified by Jing-min Zhang of the School of Pharmaceutical Sciences, Jilin University. A voucher specimen (No. 2015062001) is deposited at the Herbarium of the School of Pharmaceutical Sciences, Jilin University.

3.2. Extraction and Isolation

Two kg of air-dried whole *Hylomecon japonica* herbs were extracted twice with 20 L of 70% aqueous ethanol solution (*v/v*) at room temperature. The extraction solution was concentrated under reduced pressure to remove ethanol, and the water concentrate was filtered and then passed through a D101 polyporous resin column eluting successively with H₂O, 30% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH. The crude saponin extracts were obtained from 50% aqueous ethanol eluate by vacuum distillation recovery and used for the next experiments. The crude saponin extracts were

chromatographed on silica gel columns repeatedly eluted with EtOAc–MeOH–H₂O (7:3:0.5) and further purified by semi-preparative HPLC using acetonitrile and 0.1% formic acid solution in water as the mobile phase and the eluate was monitored at 207 nm, to yield compounds **1** (60 mg), **2** (50 mg), **3** (30 mg), and **4** (30 mg).

3.3. Characterization

Compound **1**: White amorphous solid, $[\alpha]_D^{25} +5.0^\circ$ (c 0.5, pyridine). HRESIMS, m/z : 1365.6318 [M + H]⁺; calcd for C₆₄H₁₀₁O₃₁, 1365.6327. ¹H-NMR (400 MHz, C₅D₅N) and ¹³C-NMR (100 MHz, C₅D₅N): see Table 1.

Compound **2**: White amorphous solid, $[\alpha]_D^{25} -15.0^\circ$ (c 0.5, pyridine). HRESIMS, m/z : 1351.6151 [M + H]⁺; calcd for C₆₃H₉₉O₃₁, 1351.6165. ¹H-NMR (400 MHz, C₅D₅N) δ : 0.67 (3H, s, H-25), 0.80 (3H, s, H-26), 0.87 (6H, s, H-30, H-29), 1.12 (3H, s, H-27), 1.29 (3H, s, H-24), 1.61 (3H, d, $J = 4.8$, Rha-6), 3.13 (1H, m, H-18), 3.34 (1H, t-like, $J = 10.0$ Hz, Xyl(inner)-5 α), 3.55 (1H, t-like, $J = 10.0$ Hz, Xyl(terminal)-5 α), 4.71 (1H, d, $J = 6.8$ Hz, GluA-1), 5.01 (1H, d, $J = 6.8$ Hz, Xyl(inner)-1), 5.08 (1H, d, $J = 7.2$ Hz, Gal-1), 5.10 (1H, d, $J = 7.6$ Hz, Xyl(terminal)-1), 5.29 (1H, br. s, H-12), 5.66 (1H, br. s, Rha-1), 6.34 (1H, br. s, Ara-1), 9.82 (1H, s, H-23). ¹³C-NMR (100 MHz, C₅D₅N): see Table 2.

Compound **3**: White amorphous solid, $[\alpha]_D^{25} -29.2^\circ$ (c 0.5, pyridine). HRESIMS, m/z : 1367.6101 [M + H]⁺; calcd for C₆₃H₉₉O₃₂, 1367.6114. ¹H-NMR (400 MHz, C₅D₅N) δ : 0.70 (3H, s, H-25), 0.90 (6H, s, H-26, H-29), 1.02 (3H, s, H-30), 1.28 (3H, s, H-27), 1.57 (3H, s, H-24), 1.65 (3H, d, $J = 4.8$, Rha-6), 3.44 (1H, m, H-18), 3.31 (1H, t-like, $J = 10.0$ Hz, Xyl(inner)-5 α), 3.54 (1H, t-like, $J = 10.0$ Hz, Xyl(terminal)-5 α), 4.68 (1H, d, $J = 6.8$ Hz, GluA-1), 4.98 (1H, d, $J = 6.8$ Hz, Xyl(inner)-1), 5.05 (1H, d, $J = 7.2$ Hz, Gal-1), 5.10 (1H, d, $J = 7.6$ Hz, Xyl(terminal)-1), 5.47 (1H, br. s, H-12), 5.62 (1H, br. s, Rha-1), 6.35 (1H, br. s, Ara-1), 9.76 (1H, s, H-23). ¹³C-NMR (100 MHz, C₅D₅N): see Table 2.

Compound **4**: White amorphous solid, $[\alpha]_D^{25} -10.0^\circ$ (c 0.5, pyridine). HRESIMS, m/z : 1381.6258 [M + H]⁺; calcd for C₆₄H₁₀₁O₃₂, 1381.6270. ¹H-NMR (400 MHz, D₂O–C₅D₅N) δ : 0.90 (3H, s, H-25), 0.98 (3H, s, H-26), 1.03 (3H, s, H-29), 1.04 (3H, s, H-30), 1.26 (3H, s, H-27), 1.45 (3H, s, H-24), 1.67 (3H, d, $J = 4.8$, Rha-6), 3.17 (1H, m, H-18), 3.57 (1H, t-like, $J = 10.0$ Hz, Xyl(terminal)-5 α), 4.82 (1H, d, $J = 7.2$ Hz, GluA-1), 5.09 (1H, d, $J = 8.0$ Hz, Glu-1), 5.18 (1H, d, $J = 7.6$ Hz, Gal-1), 5.29 (1H, d, $J = 8.0$ Hz, Xyl-1), 5.45 (1H, br. s, H-12), 5.57 (1H, br. s, Rha-1), 6.23 z, Ara-1), 9.78 (1H, s, H-23). ¹³C-NMR (100 MHz, D₂O–C₅D₅N): See Table 2.

3.4. Acid Hydrolysis and Stereochemistry of Sugars of **1** and **2**

Compounds **1** and **2** (each 20 mg) were hydrolyzed with 2 M HCl (5 mL) at 90 °C for 4 h. The reaction mixture was extracted with EtOAc (3 × 5 mL), washed with H₂O, and evaporated to dryness. The EtOAc extract was purified by silica gel column using a gradient of CHCl₃–MeOH (1:0–95:5) to the aglycones of **1** and **2** which were determined to be gypsogenin by comparison of their spectral data with those of an authentic sample. Aglycone: White amorphous solid; ¹³C-NMR (100 MHz, C₅D₅N) δ : 207.7 (C-23), 180.0 (C-28), 144.8 (C-13), 122.2 (C-12), 71.6 (C-3), 56.2 (C-4), 48.0 (C-5), 47.9 (C-9), 46.6 (C-17), 46.5 (C-19), 42.2 (C-14), 41.9 (C-18), 40.0 (C-8), 38.4 (C-1), 36.2 (C-10), 34.2 (C-21), 33.2 (C-29), 32.8 (C-7), 32.5 (C-22), 30.9 (C-20), 28.3 (C-15), 26.1 (C-27), 27.0 (C-2), 23.8 (C-16, C-30), 23.7 (C-11), 21.0 (C-6), 17.3 (C-26), 15.7 (C-25), 9.6 (C-24); ¹H-NMR (400 MHz, C₅D₅N) δ : 0.94 (3H, s), 0.97 (6H, s), 1.12 (6H, s), 1.23 (6H, s), 1.41 (6H, s), 3.02 (1H, m), 3.90 (1H, m), 5.29 (1H, brs), 9.66 (1H, m). The H₂O layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (2 mL), L-cysteine methyl ester hydrochloride (1.5 mg) was added, and the mixture was heated at 60 °C for 1 h. Trimethylsilylimidazole (1.5 mL) was added, and the mixture was heated at 60 °C for another 0.5 h. An aliquot (4 μ L) of the supernatant was subjected to GC analysis under the following conditions: column temp 180–280 °C at 3 deg/min, carrier gas N₂ (1 mL/min), injector and detector temp 250 °C, split ratio 1:50. The configurations of monosugars for **1** and **2** were determined by comparison of the retentions times

(t_R) of the corresponding derivatives with standard L-arabinose (t_R 10.748 min), D-xylose (t_R 11.496 min), L-rhamnose (t_R 12.162 min), D-quinovose (t_R 13.648 min), D-glucuronic acid (t_R 15.814 min) and D-galactose (t_R 18.026 min). **1** yielded D-xylose, L-rhamnose, D-quinovose, D-glucuronic acid and D-galactose, and **2** gave L-arabinose, D-xylose, L-rhamnose, D-glucuronic acid and D-galactose.

3.5. Cytotoxicity Assay

The procedure for the cytotoxic assay was performed according to the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide) reduction method. In this study, MGC-803 (human gastric cancer), HL-60 (human promyelocytic leukemia), BEL-7402 (human hepatoma carcinoma), MCF-7 (human breast cancer), and SPC-A1 (lung adenocarcinoma) cell lines were used. Compounds **1** and **2** were dissolved at a concentration of $10 \text{ mg}\cdot\text{mL}^{-1}$ in phosphate-buffered saline (PBS) (as a stock solution), filtered through a filter $0.22 \mu\text{m}$ (Millipore, Bedford, MA, USA) and stored at $4 \text{ }^\circ\text{C}$. The stock solution was diluted to 160, 80, 40, 20, $10 \mu\text{g}\cdot\text{mL}^{-1}$ before the cytotoxic assay.

Logarithmic-phase growing cells were diluted into $5 \times 10^4 \text{ cells}\cdot\text{mL}^{-1}$, seeded in 96-well plates with $100 \mu\text{L}$ per well, and incubated in RPMI 1640 medium supplemented with 10% fetal bovine serum, $100 \text{ U}\cdot\text{mL}^{-1}$ penicillin, $100 \mu\text{g}\cdot\text{mL}^{-1}$ streptomycin at $37 \text{ }^\circ\text{C}$ in a humidified atmosphere with 5% CO_2 . Blank control wells were added $100 \mu\text{L}$ of culture medium, and maintained under the same conditions. After 24 h, $100 \mu\text{L}$ of the above culture medium containing different concentrations of **1** and **2** and normal culture medium (untreated control group) were added, and incubated for 72 h. For the analysis of cytotoxicity, $50 \mu\text{L}$ ($1 \text{ mg}\cdot\text{mL}^{-1}$) XTT containing $0.15 \text{ mg}\cdot\text{mL}^{-1}$ phenazine methosulphate was added to each well, and cells were incubated for 3 h at $37 \text{ }^\circ\text{C}$. The absorbances (A) of the produced formazan were measured by a DNM-9602 enzyme immunoassay spectrophotometer at 450 nm. Three replicate wells were used for each analysis. The cell inhibitory rate (%) = $(A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{untreated}} - A_{\text{blank}}) \times 100$. The concentration of compound producing 50% of cell inhibitory rate (IC_{50}) was calculated by using SPSS version 20.0 (International Business Machines Corporation, Armonk, NY, USA).

4. Conclusions

Two new triterpenoid saponins, named hylomeconoside A (**1**) and B (**2**), along with two known triterpenoid saponins identified as dubioside C (**3**), and lucyoside P (**4**), have been isolated from an ethanolic extract of *Hylomecon japonica* herbs. It is well known that alkaloids are main constituents of the plants of *Papaveraceae* family and until now besides alkaloids, flavonoids, megastigmane derivatives, volatile oil, furan derivatives, triterpenes and sterols have also reported, but saponins have not been reported [15–17]. This is the first time that saponins have been isolated from plants of the *Papaveraceae* family. Compound **1** with the IC_{50} values of $43.8 \mu\text{g}\cdot\text{mL}^{-1}$ on MGC-803 and $32.4 \mu\text{g}\cdot\text{mL}^{-1}$ on HL-60 is considered to be one of the antitumor principles in this plant.

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Sample Availability: Samples of the compounds 1–4 are available from the authors.



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