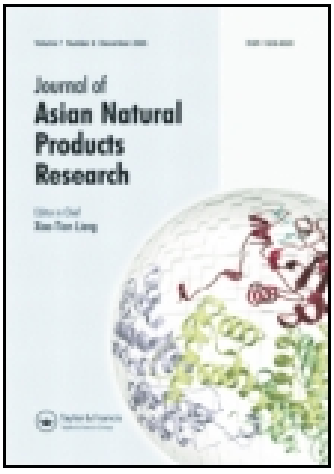

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Three new triterpenoid saponins from Ilex pubescens

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Published online: 08 Nov 2012.

To cite this article: Ling Li, Yu-Xin He, Mei-Ling Gou & Chun Dai (2012) Three new triterpenoid saponins from Ilex pubescens, Journal of Asian Natural Products Research, 14:12, 1169-1174, DOI: 10.1080/10286020.2012.738674

To link to this article: http://dx.doi.org/10.1080/10286020.2012.738674

Journal of Asian Natural Products Research
Vol. 14, No. 12, December 2012, 1169–1174

Three new triterpenoid saponins from *Ilex pubescens*

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(*Received 24 June 2012; final version received 7 October 2012*)

Three new triterpene saponins, ilexsaponins D–F (**1**–**3**), were isolated from the roots of *Ilex pubescens*. Their structures were elucidated as 3-*O*-β-D-glucopyranosyl(1 → 3)-α-L-arabinopyranosyl urs-12,18-diene-24,28-dioic acid 28-*O*-β-D-glucopyranoside (**1**), 3-*O*-β-D-glucopyranosyl(1 → 3)-α-L-arabinopyranosyl urs-12,18-diene-24,28-dioic acid (**2**), and 3-*O*-β-D-glucopyranosyl(1 → 3)-α-L-arabinoyranosyl-30-hydroxyurs-12,19-diene-24,28-dioic acid 28-*O*-β-D-glucopyranoside (**3**) on the basis of the chemical and spectroscopic evidence.

Keywords: *Ilex pubescens*; triterpene saponin; ilexsaponins D–F

1. Introduction

The roots of *Ilex pubescens* Hook et Arn. (native name, Mao-Dong-Qing) are a traditional Chinese medicine (TCM) commonly used in South China for the treatment of cardiovascular disease and hypercholesterolemia [1]. Previous phytochemical investigation of its roots and leaves led to the isolation of glaberides, triterpene saponins, and phenolics, specifically vomifoliol [2–7]. As a part of our program searching for bioactive saponins from TCMs, we present in this report the isolation and structural elucidation of three new triterpene saponins (**1**–**3**) (Figure 1) from the roots of *I. pubescens*.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. It showed positive Liebermann–Burchard and Molish reactions. The molecular formula was assigned as $C_{47}H_{72}O_{19}$ on the basis of the negative-ion HR-ESI-MS at *m/z* 939.4598 $[M-H]^-$, confirmed by its ^1H and ^{13}C NMR spectral data (Table 1). The ^1H NMR spectrum indicated six methyl group signals at δ_H 1.72, 1.17, 1.21, 1.08, 1.73 (s, 3H each), and 1.06 (3H, d, $J = 7.0$ Hz) correlated with the carbons at δ_C 24.8, 14.6, 18.7, 22.0, 19.5, and 18.6 in the HSQC spectrum, respectively. There were two carboxyl carbons at δ_C 174.7 and 180.6 and two pairs of olefinic carbon signals at δ_C 126.7, 138.8, 133.8, and 136.0 in the ^{13}C NMR spectrum. An olefinic proton at δ_H 5.71 (brt) correlated with the carbon at δ_C 126.7 in the HSQC spectrum (see Table 1) indicated the presence of an ursane-type triterpene aglycon. The UV absorbance at 225 nm suggested that compound **1** contains a heteroannular-conjugated diene system [8]. The second double bond located at C-18/C-19 was assigned by the interactions of the olefinic proton at δ_H 5.71 (brt, $J = 3.0$ Hz), methyl protons H-29 (δ_H 1.73) with the carbon signals C-18 (δ_C 133.8), and methyl protons H-29 (δ_H 1.73), H-30 (δ_H 1.06) with C-19 (δ_C 136.0) in the HMBC spectrum. In addition, the HMBC correlations of H-23 (δ_H 1.72), H-3

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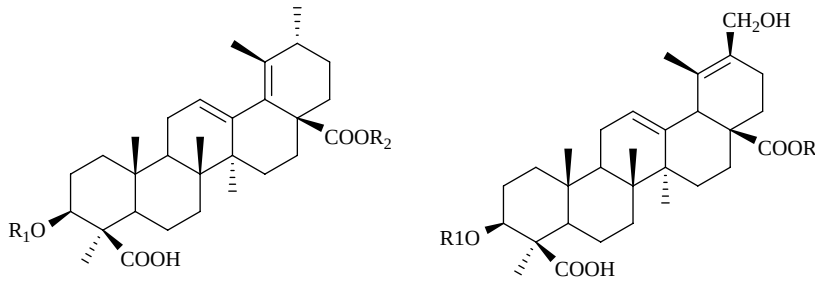
ISSN 1028-6020 print/ISSN 1477-2213 online

http://dx.doi.org/10.1080/10286020.2012.738674
http://www.tandfonline.com



	R_1	R_2
1	Ara^3-Glc	Glc
2	Ara^3-Glc	H

	R_1	R_2
3	Ara^3-Glc	Glc

Figure 1. The structures of compounds **1**–**3**.

(δ_H 3.34) with C-24 (δ_C 180.6) showed that the carboxyl carbon at δ_C 180.6 was located at C-24, therefore the other carboxyl carbon at δ_C 174.7 was attributed to C-28 (see Figure 2). After extensive NMR (^{1}H NMR, ^{13}C NMR, HMBC, and NOESY) spectral analysis (Table 1), the aglycon was established to be a 3β-hydroxyurs-12, 18-dien-24, 28-dioic acid [9]. ESI-MS showed the ion peaks at *m/z* 777 $[M-H-162]^-$, 483 $[M-H-162-132-162]^-$, attributable to the sequential losses of a hexose, pentose, and hexose residues. Acid hydrolysis and gas chromatography (GC) experiment of **1** showed that the sugar moieties are L-arabinose and D-glucose [10]. H-1′ (δ_H 4.95) of the L-arabinose was correlated with the C-3 (δ_C 88.9), and H-1″ (δ_H 5.16) of the terminal glucose was correlated with C-3′ (δ_C 81.1) of L-arabinosyl in the HMBC spectrum. H-1‴ (δ_H 6.28) of the D-glucose was correlated with the C-28 (δ_C 174.7) in the HMBC spectrum. The anomeric configuration of the L-arabinosyl and D-glucosyl moieties were α and β, respectively, from the $^3J_{1\text{-}2}$ coupling constant and NOESY experiment [10]. On the basis of this evidence, compound **1** was characterized as 3-*O*-β-D-glucopyranosyl(1 → 3)-α-L-arabinopyranosyl urs-12,18-diene-24,28-dioic acid 28-*O*-β-D-glucopyranoside, named ilexsaponin D.

Compound **2** was obtained as a white amorphous powder. It showed positive Liebermann–Burchard and Molish reactions. The molecular formula was assigned as $C_{41}H_{62}O_{14}$ on the basis of the negative-ion HR-ESI-MS at *m/z* 777.4033 $[M-H]^-$. Acid hydrolysis of **2** afforded L-arabinose, D-glucose, and the aglycon, which is the same as those of **1**. Comparison of the NMR spectral data for **2** and **1** revealed that the two compounds are similar except that signals due to the D-glucopyranoside linked to C-28 in **1** were absent for **2**. This observation was supported by a relative downfield shift of C-28 (δ_C 180.6) of **2** in the ^{13}C NMR spectrum. Thus, the structure of **2** was determined as 3-*O*-β-D-glucopyranosyl (1 → 3)-α-L-arabinopyranosyl urs-12,18-diene-24,28-dioic acid, named ilexsaponin E.

Compound **3** was obtained as a white amorphous powder. It showed positive Liebermann–Burchard and Molish reactions. The molecular formula was assigned as $C_{47}H_{72}O_{20}$ on the basis of the negative-ion HR-ESI-MS at *m/z* 955.4539 $[M-H]^-$. In the ^{1}H NMR spectrum, the aglycon part of **3** showed five methyl proton signals δ_H 1.67, 1.11, 1.19, 1.12, and 1.83 (s, 3H each), and one olefinic proton signal at δ_H 5.72 (brt). It also had two pairs of olefinic carbon signals at δ_C 128.1, 129.7, 131.1, and 137.6

Table 1. ^{1}H and ^{13}C NMR spectral data for 1–3 (500 MHz for ^{1}H and 125 MHz for ^{13}C, in C_5D_5N, δ in ppm, J in Hz).[a]

	1		**2**		**3**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	39.2	1.03 m	39.1	1.00 m	39.7	1.06 m
		1.81 m		1.82 m		1.70 m
2	28.3	1.21 m	28.7	1.20 m	28.2	1.98 m
		2.00 m		2.00 m		
3	88.9	3.34 dd (14.0, 4.0)	88.9	3.33 dd (14.0, 4.0)	88.7	3.31 dd (14.0, 4.0)
4	48.2		48.7		48.0	
5	57.1	1.04 m	57.0	1.07 m	57.0	1.06 m
6	20.8	2.00 m	20.8	1.98 m	20.7	1.99 m
		2.14 m		2.10 m		2.09 m
7	35.3	1.52 m	35.3	1.55 m	34.5	1.53 m
		2.58 m		2.44 m		
8	39.4		39.5		39.7	
9	47.6	1.52 m	47.4	1.55 m	47.8	1.57 m
10	37.7		37.7		37.8	
11	23.8	2.00 m	23.8	2.02 m	24.0	1.98 m
12	126.7	5.71 brt (3.0)	126.3	5.73 brt (3.0)	128.1	5.72 brt (3.0)
13	138.8		138.9		137.6	
14	45.0		45.1		43.7	
15	28.9	1.25 m	28.9	1.24 m	28.8	1.23 m
		2.42 m		2.41 m		2.22 m
16	35.4	1.50 m	35.6	1.49 m	23.6	2.03 m
		2.55 m		2.55 m		
17	49.9		49.9		47.3	
18	133.8		133.7		51.8	3.63 s
19	136.0		136.0		131.1	
20	34.6	2.14 m	34.7	2.14 m	129.7	
21	26.8	1.25 m	26.8	1.23 m	24.4	2.56 m
		2.07 m		2.07 m		
22	31.0	1.68 m	31.2	1.68 m	33.0	1.85 m
		2.14 m		2.13 m		2.14 m
23	24.8	1.72 s	24.9	1.71 s	24.7	1.67 s
24	180.6		180.4		180.9	
25	14.6	1.17 s	14.5	1.18 s	14.4	1.11 s
26	18.7	1.21 s	18.8	1.21 s	18.3	1.19 s
27	22.0	1.08 s	22.1	1.09 s	22.2	1.12 s
28	174.7		180.6		176.2	
29	19.5	1.73 s	19.5	1.76 s	16.8	1.83 s
30	18.6	1.06 d (7.0)	18.7	1.07 d (7.0)	63.0	4.39, 4.46 d (6.5)
3-*O*-sugar-Ara						
1′	104.8	4.95 d (5.5)	105.0	4.96 d (5.5)	104.8	4.93 d (5.5)
2′	73.4	4.57 m	73.4	4.55 m	73.4	4.55 m
3′	81.1	4.23 m	81.3	4.23 m	81.1	4.20 m
4′	68.3	4.34 m	68.5	4.33 m	68.3	4.35 m
5′	64.9	3.76 m	64.7	3.76 m	64.9	3.74 m
		4.26 m		4.25 m		4.27 m
Glc						
1″	106.0	5.16 d (7.5)	106.2	5.20 d (7.5)	106.0	5.11 d (7.5)
2″	76.4	4.07 m	76.7	4.07 m	76.4	4.05 m
3″	78.1	4.17 m	78.3	4.16 m	78.1	4.16 m
4″	71.5	4.31 m	71.7	4.33 m	71.5	4.32 m
5″	78.1	3.80 m	78.3	3.83 m	78.1	3.77 m

Table 1 – *continued*

	1		2		3	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
6″	62.5	4.38 m 4.41 m	62.6	4.38 m 4.42 m	62.5	4.37 m 4.42 m
28-*O*-sugar-Glc						
1‴	95.8	6.28 d (8.0)			95.8	6.35 d (8.0)
2‴	74.1	4.18 t (8.5)			74.1	4.22 m
3‴	78.8	4.25 t (8.9)			78.9	4.30 m
4‴	71.1	4.31 t (9.2)			71.3	4.42 m
5‴	79.2	4.00 m			79.0	4.12 m
6‴	62.2	4.37 m 4.42 m			62.4	4.41 m 4.54 m

[a] The assignments were based upon ^{1}H NMR, ^{13}C NMR, HSQC, HMBC, and NOESY spectra.

in the ^{13}C NMR spectrum. UV absorbance at 203 nm of **3** suggested that there was no conjugation between the two double bonds. The double bond was located at C-12/C-13 (δ_C 128.1 and 137.6) according to the characteristic proton signal at δ_H 3.63 (1H, s, H-18) and carbon signal δ_C 51.8 (C-18) [9]. The correlations of H-29 (δ_H 1.83) with C-19 (δ_C 131.1) and C-20 (δ_C 129.7) in the HMBC experiment suggested the second double bond was located at C-19/C-20. The hydroxymethyl C-30 was attached to C-20 based on the following HMBC correlations of H-30 (δ_H 4.39 and 4.46) with C-19 (δ_C 131.1), C-20 (δ_C 129.7), and C-21 (δ_C 24.4). In addition, the two carbonyls at C-24 and C-28 were assigned by the HMBC correlations of H-23 (δ_H 1.67), H-3 (δ_H 3.31) with C-24 (δ_C 180.9) and H-18 (δ_H 3.63) with C-28 (δ_C 176.2). The aglycon moiety was further confirmed to be olean-12, 19-diene-24, 28-dioic acid by comparison with the literature data [9]. Acid hydrolysis of **3** also afforded L-arabinose and D-glucose. Detailed NMR spectral comparison of the sugar moiety for **3** with **1** (see Table 1) demonstrated that both compounds possess same sugar sequence. Consequently, compound **3** was elucidated to be 3-*O*-β-D-glucopyranosyl(1 → 3)-*α*-L-arabinoyranosyl-30-hydroxyurs-12,19-diene-24,28-dioic acid 28-*O*-β-D-glucopyranoside, named ilexsaponin F.

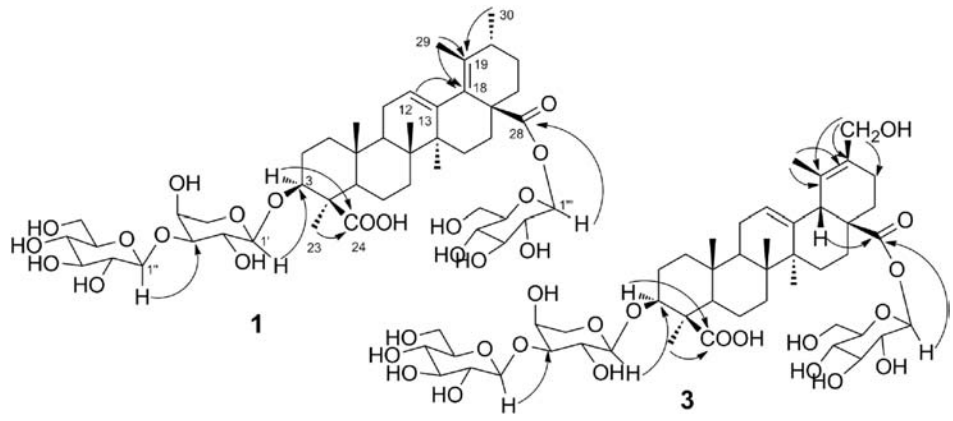


Figure 2. Selected HMBC correlations for compounds **1** and **3**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter (JASCO Corporation, Tokyo, Japan). Spots were visualized by spraying 10% H_2SO_4–EtOH followed by heating. UV spectra were obtained on a TU-1901 spectrometer (Beijing Purkinje General Instrument Co., Ltd, Beijing, China). IR spectra were obtained on a Perkin-Elmer 577 spectrometer (PerkinElmer, Waltham, MA, USA). NMR spectra were recorded on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA), operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ESI-MS data were recorded on an MS Agilent 1100 series LC/MSD Trap Mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) and HR-ESI-MS data were obtained on a G1969A TOF-MS instrument (Agilent Technologies Inc.). Precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Qingdao, China) were employed for thin layer chromatography. Column chromatography was carried out on silica gel (Qingdao Haiyang Chemical Co.) and macroporous resin D_{101} (pore size B 13–14 nm, 26–60 mesh; Tianjin, China). High performance liquid chromatography (HPLC) separation was carried out on an ODS column (YMC-pack ODS-A, 250 × 10 mm, i.d. 5 μm, YMC, Kyoto, Japan) with an Alltech evaporative light scattering detector (Alltech, Dearfield, IL, USA). GC analysis was carried out on an Agilent 6890N gas chromatograph (Agilent, Waldbronn, Germany) using a HP-5 capillary column (28 m × 0.32 mm i.d.), with an FID detector (detector temperature: 260°C; column temperature: 180°C; carrier gas: N_2; flow rate: 40 ml/min).

3.2 Plant material

The roots of *I. pubescens* were collected in Guangzhou, Guangdong Province, China, in June 2010, and identified by Prof. Y.-X. He, Xihua University. A voucher specimen (No. 20100810) has been deposited in the herbarium of Bioengineering College of Xihua University.

3.3 Extraction and isolation

The roots of *I. pubescens* (5 kg) were refluxed two times with 70% (v/v) EtOH, each for 2 h. After concentrated *in vacuo*, the residue was suspended in water, and partitioned with ethyl acetate and *n*-butanol successively. The *n*-butanol fraction (114 g) was further chromatographed over a macroporous resin D_{101} column eluted initially with water, then with 30%, 50%, and 70% EtOH to give fractions A–C. Fraction B (34 g) was subjected to a silica gel column using $CHCl_3$–MeOH–H_2O (90:10:0.1–70:30:5) as an eluent to give fractions B_1–B_9. Fraction B_3 (2.9 g) was subjected to ODS open column chromatography (MeOH–H_2O 40:60 to 80:20) to afford fractions $B_{3\text{-}1}$–$B_{3\text{-}4}$. Fraction $B_{3\text{-}1}$ (600 mg) was subjected to prep-HPLC (MeOH–H_2O, 40:60, UV detection at 210 nm, affording **1** (18 mg, t_R 16.7 min), **2** (12 mg, 20.7 min), and **3** (5 mg, 18.5 min), respectively.

3.3.1 Ilexsaponin D (1)

White amorphous powder; $[\alpha]_D^{22} + 32$ ($c = 0.12$, MeOH); UV (MeOH) λ_{max} 225 nm; IR (KBr) ν_{max} (cm^{-1}): 3432, 1724, 1681. ^{1}H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 939 $[M-H]^-$; HR-ESI-MS *m/z* 939.4598 $[M-H]^-$ (calcd for $C_{47}H_{71}O_{19}$, 939.4595).

3.3.2 Ilexsaponin E (2)

White amorphous powder; $[\alpha]_D^{30} + 34$ ($c = 0.12$, MeOH); UV (MeOH) λ_{max} 225 nm; IR (KBr) ν_{max} (cm^{-1}): 3432, 1725, 1680. ^{1}H NMR(C_5D_5N, 500 MHz) and ^{13}C NMR(C_5D_5N, 125 MHz) spectral

data, see Table 1; ESI-MS: *m/z* 777 $[M-H]^-$; HR-ESI-MS *m/z* 777.4033 $[M-H]^-$ (calcd for $C_{41}H_{61}O_{14}$, 777.4066).

*3.3.3 Ilexsaponin F (**3**)*

White amorphous powder; $[\alpha]_D^{30} + 40$ ($c = 0.12$, MeOH). UV (MeOH) λ_{max} 203 nm; IR (KBr) ν_{max} (cm^{-1}): 3432, 1725, 1683. ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectral data, see Table 1; ESI-MS: *m/z* 955 $[M-H]^-$; HR-ESI-MS *m/z* 955.4539 $[M-H]^-$ (calcd for $C_{47}H_{71}O_{20}$, 955.4544).

3.4 Acid hydrolysis of 1–3

Each saponin (5 mg) was heated in 5 ml of 10% HCl–dioxane (1:1) at 80°C for 4 h. After the dioxane was removed, the solution was extracted with EtOAc (3 ml × 3) to yield the aglycon and the sugar, respectively. The sugar components in the aqueous layer obtained after acid hydrolysis of **1**–**3** were analyzed by GC analysis of the methyl sugar peracetates. The aqueous layer was evaporated and dissolved in anhydrous pyridine (100 ml), 0.1 M L-cysteinemethyl ester hydrochloride (200 ml) was added, and the resultant was warmed at 60°C for 1 h. The trimethylsilylation reagent HMDS–TMCS (hexamethyldisilazane–trimethylchlorosilane–pyridine, 2:1:10; Acros Organics, Geel, Belgium) was added and warmed at 60°C for 30 min. To the mixture, the thiazolidine derivatives were analyzed by GC for sugar identification. The retention times of L-arabinose (t_R, 5.32 min) and D-glucose (t_R, 12.39 min) were confirmed by comparison with those of authentic standards [11].

Acknowledgments

This work was financially supported by the Youth Fund of Sichuan Province Office of Education (08Z6012), the Chun Hui Project of Ministry of Education (Z2010103), and the Innovation Fund of Postgraduate, Xihua University (YCJJ201243).

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