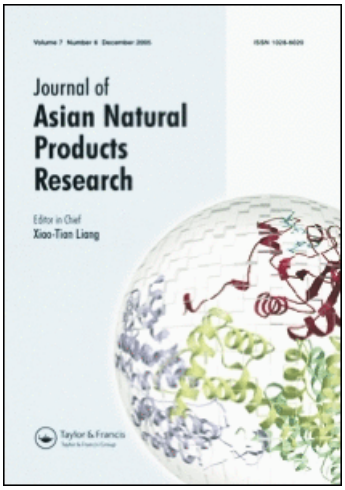

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Feng, FENG , Zhu, MING-XIAO , Xie, NING , Liu, WEN-YUAN , Chen, DONG-JUN and You, QI-DONG(2008) 'Two new triterpenoid saponins from the root of *Ilex pubescens*', Journal of Asian Natural Products Research, 10: 1, 71 — 75
To link to this Article: DOI: 10.1080/10286020701273874
URL: http://dx.doi.org/10.1080/10286020701273874

Journal of Asian Natural Products Research, Vol. 10, No. 1, January 2008, 71–75

Two new triterpenoid saponins from the root of *Ilex pubescens*

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(Received 28 August 2006; revised 9 January 2007; in final form 18 January 2007)

Two new triterpenoid saponins, named ilexsaponins B_4 (**1**) and C (**2**), have been isolated from the roots of *Ilex pubescens*. Their structures have been established as ilexgenin B 3-*O*-α-L-arabinopyranosyl-(2 → 1)-β-D-glucopyranosyl-(2 → 1)-β-D-xylopyranoside (**1**) and 3-*O*-β-D-xylopyranosyl spathodic acid 28-β-D-glucopyranosyl ester (**2**) by means of spectral and chemical methods.

Keywords: Triterpenoid saponin; *Ilex pubescens*; Ilexsaponin B_4; Ilexsaponin C

1. Introduction

"Mao-dong-qing", the dried roots of *Ilex pubescens* Hook. *et* Arn., is widely distributed in the south of China. It has the effect of activating blood circulation and stimulating menstrual discharge, removing blood stasis, relieving pain and clearing away heat and toxin. It is widely used for the treatment of coronary heart diseases, angina pectoris and vasculitis. Several kinds of compounds, i.e. flavonoids, coumarins, terpenoids, saccharides, and amino acids, were isolated from it [1]. We investigated the chemical constituents of the roots of this plant and isolated nine compounds, whose structures were elucidated by physical and chemical means (UV, IR, 1D NMR, 2D NMR, MS). Two triterpenoid saponins are proved to be new compounds, named ilexsaponins B_4 (**1**) and C (**2**). Moreover, the compound magnolenin C [2] was isolated from the genus of *Ilex* for the first time and two compounds, acanthoside B and liriodendin [3], were isolated from this plant for the first time. The other four known compounds are ilexsaponin B_1, ilexsaponin B_2 [4], myo-inositol [5], and ilexsoside O [6], respectively.

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Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2008 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020701273874

2. Results and discussion

Compound **1** was obtained as colourless needles, mp 241–243°C (CH_3OH), and gave positive results for the Liebermann–Burchard test and Molish test. HRESI-MS showed $[M - H]^-$ at *m/z* 897.4836, corresponding to the molecular formula $C_{46}H_{74}O_{17}$. Its ^{13}C NMR and DEPT spectra revealed 46 carbon signals ($CH_3 \times 7$, $CH_2 \times 12$, $CH \times 19$, $C \times 8$). Its IR spectrum showed absorption bands at 3475 cm^{-1} for hydroxyl groups, 1679 cm^{-1} for a carboxyl group and 1639 cm^{-1} for a double bond. The ESI-MS showed a quasi-molecular ion peak at *m/z* 897 $[M - H]^-$ and fragments at *m/z* 765 $[M - H - 132]^-$, 603 $[M - H - 132 - 162]^-$, indicating the compound was a triterpenoid saponin. The ^1H NMR spectrum revealed six methyl singlets at δ 0.88, 1.08, 1.08, 1.24, 1.43, 1.74 and a methyl doublet at δ 1.12 (3H, d, $J = 7.1$ Hz, 30-Me), as well as an olefinic proton at δ 5.55 (1H, br t, $J = 3.2$ Hz). The ^{13}C NMR spectrum showed seven methyl signals at δ 15.5, 16.1, 16.8, 17.3, 24.4, 28.2, and 29.8. The olefinic carbon signals at δ 127.3 and 139.5 were attributed to C-12 and C-13, respectively. These spectral data indicated that **1** has an urs-12-en-skeleton. In fact, the ^{13}C NMR spectrum of **1** was very similar to that of ilexgenin B, except that C-3 at δ 88.7 was shifted downfield for 10.5 ppm compared with that of ilexgenin B [4], indicating the sugar chain was attached to C-3. The final identification of all NMR signals was carried out by ^1H—^1H COSY, HSQC, and HMBC experiments, taking the assignment of ilexgenin B as a reference [4]. The trisaccharide nature of **1** was verified by its ^1H NMR [δ 4.89 (1H, d, $J = 6.8$ Hz), 5.48 (1H, d, $J = 7.7$ Hz), 5.39 (1H, d, $J = 7.0$ Hz)] and ^{13}C NMR (δ 105.2, 103.2, 106.5) data. On acid hydrolysis, **1** gave the same spots as xylose, glucose, and arabinose on high-performance thin-layer chromatography (HPTLC). In the HMBC spectrum, the cross peaks between H-1 (δ 4.89) of xylose and C-3 (δ 88.7) of the aglycone, H-1 (δ 5.48) of glucose and C-2 (δ 82.6) of xylose, H-1 (δ 5.39) of arabinose and C-2 (δ 84.6) of glucose were easily observed, and the structure of **1** was thus confirmed. The anomeric configurations of all sugars could be determined by the coupling constants of the anomeric protons and ^{13}C NMR spectral data [5]. Thus, the structure of **1** was established as ilexgenin B 3-*O*-α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-β-D-xylopyranoside.

Compound **2** was obtained as colourless needles, mp 230–231°C (CH_3OH), and gave positive results for the Liebermann–Burchard test and Molish test. HRESI-MS showed $[M - H]^-$ at *m/z* 781.4361, corresponding to the molecular formula $C_{41}H_{66}O_{14}$. Its ^{13}C NMR and DEPT spectra revealed 41 carbon signals. Its IR spectrum showed absorption bands at 3454 cm^{-1} for hydroxyl groups, 1729 cm^{-1} for an ester bond and 1639 cm^{-1} for a double bond. The ESI-MS spectrum showed a quasi-molecular ion peak at *m/z* 781 $[M - H]^-$ and fragments at *m/z* 619 $[M - H - 162]^-$, 601 $[M - H - 162 - 18]^-$, 469 $[M - H - 162 - 18 - 132]^-$, indicating that the compound might be a triterpenoid saponin. The ^1H NMR spectrum revealed the presence of six methyl singlets at δ 0.85, 0.99, 1.12, 1.15, 1.54, and 1.62, and an olefinic proton at δ 5.49 (1H, br t, $J = 3.4$ Hz). The ^{13}C NMR spectrum revealed six methyl carbon signals at δ 15.2, 17.2, 23.2, 24.5, 24.6, and 27.8. The olefinic carbon signals at δ 123.8 and 144.0 were attributed to C-12 and C-13, respectively. These spectral data indicated that **2** has an olean-12-en-skeleton. Comparison of the ^{13}C NMR spectral data of **2** with those of ilexoside XLVII (table 1) [7] revealed that **2** has the similar spectra to ilexoside XLVII, indicating the aglycon of **2** is the same as that of ilexoside XLVII. The final identification of all NMR signals was carried out by ^1H—^1H COSY, HSQC, and HMBC experiments, taking the assignment of ilexoside XLVII as

Table 1. ^{13}C-NMR spectral data of compounds **1** and **2** (125 MHz, in pyridine-d_5, δ in ppm).

C	***1***	***2***	*C*	***1***	***2***
1	38.8	38.2	26	17.3	17.2
2	27.1	26.8	27	24.4	24.5
3	88.7	88.7	28	180.6	177.0
4	39.6	44.1	29	29.8	27.8
5	55.9	56.1	30	16.8	24.6
6	18.7	18.8	3-O-	xyl	xyl
7	33.6	33.3	1	105.2	106.6
8	40.3	39.9	2	82.6	75.1
9	47.7	47.9	3	77.8	78.3
10	37.0	36.6	4	71.7	70.9
11	24.9	24.3	5	66.5	66.9
12	127.3	123.8		glc	28-O-glc
13	139.5	144.0	1	103.2	95.7
14	42.2	41.9	2	84.6	73.9
15	29.3	28.5	3	77.9	78.7
16	26.6	28.7	4	70.7	71.0
17	47.9	46.3	5	77.8	79.0
18	47.4	44.4	6	62.7	62.0
19	73.5	80.9		ara	
20	43.0	35.3	1	106.5	
21	24.0	28.8	2	72.7	
22	32.4	32.8	3	75.9	
23	28.2	23.2	4	70.5	
24	15.5	63.1	5	67.4	
25	16.1	15.2			

a reference [6]. The bisaccharide nature of **2** is verified by its ^{1}H NMR [δ 4.93 (1H, d, $J = 7.4$ Hz), 6.37 (1H, d, $J = 8.1$ Hz)] and ^{13}C NMR (δ 106.6, 95.7) data. After acid hydrolysis, **2** gave the same spots as xylose and glucose on high-performance thin-layer chromatography (HPTLC). In the HMBC spectrum (figure 1), the cross peaks between H-1 (δ 4.93) of xylose and C-3 (δ 88.7) of the aglycone, H-1 (δ 6.37) of glucose and C-28 (δ 177.0) of the aglycone were easily observed, and the structure of **2** was thus confirmed. The anomeric configurations of all sugars could be determined by their coupling constants

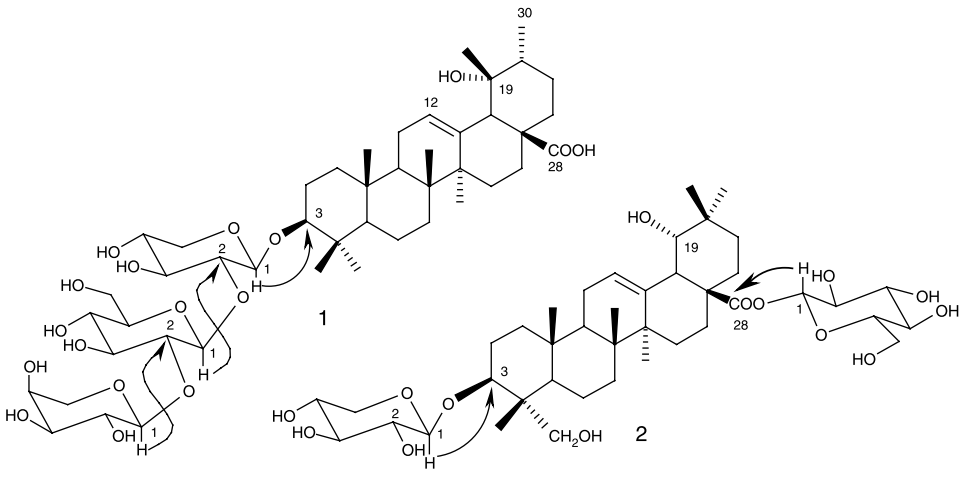

Figure 1. The structures of compounds **1** and **2**.

of the anomeric protons and ^{13}C NMR spectral data [5]. Thus, the structure of **2** was established as 3-*O*-β-D-xylopyranosyl spathodic acid 28-β-D-glucopyranosyl ester.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 micromelting apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241MC polarimeter. IR spectra were obtained on Shimadzu-FTIR-8400S IR spectrometer. NMR spectra were recorded on a Bruck AM-500 (^1H, 500 MHz; ^{13}C, 125 MHz) spectrometer. ESI-MS/MS was performed with Angilent 1100 LC/MSD. For column chromatography, silica gel (200–300 mesh, Qingdao), ODS (45–75 μm, Alltech), and Sephadex LH-20 (Pharmacia) were used.

3.2 Plant materials

The roots of *Ilex pubescens* Hook. *et* Arn., "Mao-dong-qing", were collected in July 2004 at Jiangxi Province, China and were identified by Ning Xie. The voucher specimen was identified by the authors, and has been deposited at the Department of Phytochemistry, China Pharmaceutical University.

3.3 Extraction and isolation

Dried roots (8 kg) were crushed and extracted with 95% EtOH (3 × 3 L) under reflux, and the combined extracts were concentrated, then the resulting extract (410 g) was suspended in water and successively extracted with petroleum ether, ethyl acetate, and *n*-butanol saturated with water to give the respective extracts after solvent removal. The *n*-butanolic portion (180 g) was subjected to column chromatography on silica gel with $CHC_{l3}/CH_3OH/H_2O$ (10:4:0.2) to give six fractions (Fractions I–VI). Fraction III was separated on a silica gel column, eluted with $CHCl_3/CH_3OH/H_2O$ (10:1:0–10:3:0.2), and then subjected repeatedly to an ODS column (45–65% CH_3OH), purified with Sephadex LH-20 to give compounds **1** (17 mg) and **2** (14 mg) successively. Through similar ways, the other seven known compounds, acanthoside B (from fraction I), ilexsaponin B_1, magnolenin C and liriodendin (from fraction II), ilexsaponin B_2 (from fractions III–IV), myo-inositol, and ilexsoside O (from fraction V) were isolated.

3.3.1 Ilexsaponins B_4 (1). Colourless needles, mp 241–243°C (CH_3OH). $[\alpha]_D^{30} + 15$(*c* 0.13, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3475, 2946, 1679, 1639, 1452, 1384, 1074. ^{13}C NMR and ^1H NMR (in pyridine-d_5, TMS) see tables 1 and 2, respectively. HRESI-MS *m/z* 897.4836 $[M - H]^-$ (calcd for $C_{46}H_{74}O_{17}$, 897.4847). ESI-MS *m/z* 897 $[M - H]^-$, 765 $[M - H - 132]^-$, 603 $[M - H - 132 - 162]^-$.

3.3.2 Ilexsaponins C (2). Colourless needles, mp 230–231°C (CH_3OH). $[\alpha]_D^{30} + 7$ (*c* 0.09, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3454, 2945, 1729, 1639, 1452, 1383, 1163, 1073. ^{13}C NMR and ^1H NMR (in pyridine-d_5, TMS) see tables 1 and 2, respectively. HRESI-MS *m/z* 781.4361

Table 2. ^{1}H NMR data of compounds **1** and **2** (500 MHz, in pyridine-d_5, δ in ppm, J in Hz).

H	*1*	*2*	*H*	*1*	*2*
1	0.93 m	0.92 m	25	1.08 s (3H)	0.85 s (3H)
	1.52 m	1.47 m	26	1.08 s (3H)	1.12 s (3H)
2	3.21 m	1.38 m	27	1.74 s (3H)	1.62 s (3H)
	2.07 m†	2.12 m	29	1.43 s (3H)	1.15 s (3H)
3	3.24 dd, 11.6 4.1	3.54 dd 11.2, 4.0	30	1.12 d 7.1 (3H)	0.99 s (3H)
5	0.79 br d, 12.0	0.98 m	3-O-	xyl	xyl
6	1.51 m	1.31m	1	4.89 (d, 6.8)	4.93 (d, 7.4)
	1.33 m	1.39 m	2	4.09 m	3.97 m
7	1.57 m	1.45 m	3	4.26 m	4.16 m†
	1.37 m	1.59 m	4	4.23 m	4.35 m†
9	1.82 m	1.79 m	5	4.32 m, 3.69 m	3.78 m, 4.39 m
11	2.01 m	1.69 m		glc	28-O-glc
	1.32 m	1.62 m	1	5.48 (d, 7.7)	6.37 (d, 8.1)
12	5.55 br t 3.2	5.49 br t 3.4	2	4.18 m	4.22 m
15	2.30 m (2H)	2.36 m	3	4.28 m	4.38 m†
		2.46 m			
16	2.07 m†	2.85 m	4	4.12 m†	4.32 m†
	1.87 m	1.20 m	5	3.85 m	4.03 m
18	3.30 br s	3.52 br.s	6	4.43 m, 4.37 m	4.41 m, 4.16 m
19		3.58 m		ara	
20	2.01 m		1	5.39 (d, 7.0)	
21	2.02 m	1.23 m	2	4.20 m†	
	1.72 m	1.28 m	3	4.11 m†	
22	1.96 m	2.05 m	4	4.13 m†	
	2.25 m	1.95 m	5	3.72 m, 4.22 m†	
23	1.24 s (3H)	1.54 s (3H)			
24	0.88 s (3H)	4.38 m			
		3.63 dd, 11.7, 4.9			

† Overlap signals.

$[M - H]^-$ (calcd for $C_{41}H_{66}O_{14}$, 781.4374). ESI-MS m/z 781 $[M - H]^-$, 619 $[M - H - 162]^-$, 601 $[M - H - 162 - 18]^-$, 469 $[M - H - 162 - 18 - 132]^-$.

Acknowledgements

The authors are grateful to Mr. De-Ran Xu (China Pharmaceutical University) for recording the ESI-MS/MS spectra.

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