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Triterpene saponins with XOD inhibitory activity from the roots of *Ilex pubescens*

Li Ping Lin, Wei Qu, Jing Yu Liang *

Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

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Abstract

A new triterpene saponin with 28-*nor*-urs-12(13),18(17)-dien-3β-ol as aglycone, named ilexsaponin C (**1**) was isolated from the roots of *Ilex pubescens*, together with three known saponins **2**, **3** and **4**. The structure of **1** was elucidated on the basis of spectral analysis including 1D and 2D NMR and HR-ESI-MS. Saponins **1** and **4** exhibited significant XOD inhibitory activity in the test.

Keywords: Triterpene saponin; *Ilex pubescens*; Ilexsaponin C; Xanthine oxidase

The leading function of xanthine oxidase (XOD) is to catalyze the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. The overaccumulation of uric acid can lead to hyperuricemia, which can be linked to gout because of the deposition of uric acid in the joints leading to painful inflammation. Thus, the XOD inhibitor that blocks the synthesis of uric acid in the body should be one of the approaches for the treatment of hyperuricemia. Many scientists have turned their interest to explore XOD inhibitor from a wide variety of traditional herbal plants. Therefore, we pay attention to study the plant, *Ilex pubescens* Hook. et ARN. (Aquifoliaceae), which is an evergreen bush with a wide distribution in Southern China, because its root was used as a traditional Chinese medicine for the treatment of cardiovascular diseases, hypercholesteremia and other inflammatory diseases, which may relate to hyperuricemia. Previous phytochemical investigations on *I. pubescens* revealed this plant was a rich source of triterpene saponins [1–4] that were the main active components contributing to the therapeutic effects [1,5,6]. As part of research for the bioactive compounds from *I. pubescens*, we herein describe the isolation and structure elucidation of a new triterpene saponin, named Ilexsaponin C (**1**) (Fig. 1), as well as three known saponins **2**–**4**.

The roots of *I. pubescens* were collected from Fujian Province, P.R. China (2008) and identified by Prof. Xue-Hua Song, the curator of TCM Specimen Hall of China Pharmaceutical University.

The roots of *I. pubescens* (9.6 kg) were crushed and then extracted with 70% EtOH, followed by 50% EtOH at 70 °C. The combined extract was evaporated *in vacuo* gave 486 g precipitate which was further separated by column chromatography (SiO_2, PE/EtOAc, EtOAc/MeOH) to afford six fractions (Fr.1–6). Fr. 6 (60 g) was subsequently subjected to repeated silica gel, ODS, and Sephadex LH-20, yielding compounds **1** (6.3 mg), **2** (105 mg), **3** (120 mg) and **4** (11 mg).

* Corresponding author.
E-mail address: jyliang08@126.com (J.Y. Liang).

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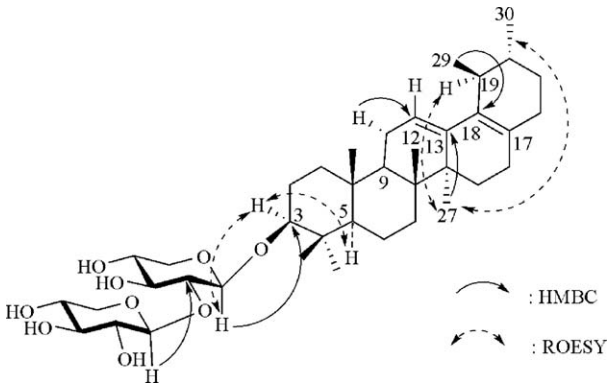

Fig. 1. The key HMBC (H → C) and ROESY correlations of the compound **1**.

Ilexsaponin C (**1**) was obtained as a white amorphous powder. $[\alpha]_D^{20}$ −36.2 (*c* 0.02, MeOH). Positive results of both Liebermann-Burchard and Molish reactions indicated that **1** could be a triterpenoid saponin. The HR-ESI-MS of **1** exhibited a *quasi*-molecular ion $[M+Na]^+$ at *m/z* 727.4378 (calcd. 727.4392), consistent with a molecular formula $C_{40}H_{64}O_{10}$. The UV maximum absorption at 237 nm (MeOH) and the double bonds peak (1635 cm^{-1}) in its IR (KBr) spectrum indicated the existence of a conjugated diene system.

The ^1H NMR of the aglycone portion showed signals of five tertiary methyl groups at δ 1.27, 1.10, 0.92, 0.84, 1.02, two secondary methyl groups at δ 1.02 (d, 3H, J = 5.5 Hz), 0.93 (d, 3H, J = 7.5 Hz) and an olefinic proton at δ 5.64 (*t*-like, 1H), which indicated the compound **1** was an ursane derivative [7]. The ^{13}C NMR and DEPT spectra exhibited 40 carbon signals, 29 of which were assigned to the aglycone part. Among them, four characteristic olefinic C-atom signals at δ 117.5, 138.2, 130.2 and 128.2 were observed, which formed conjugated diene confirming through ^1H–^1H COSY and HMBC analysis (Fig. 1). The ^1H–^1H COSY correlation from H-9 [δ 1.56 (dd, J = 5.5, 11.0 Hz)] to H-11 (δ 1.98, m) and HMBC spectrum correlations between H-11 and C-12 (δ 117.5), Me-27 (δ 1.02, s) and C-13 (δ 138.2) suggested that one double bond should be assigned as C-12 and C-13. Similarly, another double bond was deduced to

Table 1
^1H NMR (500 MHz) and ^{13}C NMR (125 MHz), DEPT and HMBC data for **1** (C_5D_5N, δ in ppm, J in Hz).

No.	δ_C (DEPT)	δ_H	HMBC	No.	δ_C (DEPT)	δ_H	HMBC
1	39.1 (CH_2)	0.94 (d, 7.5), 1.62 (m)	H-25	21	23.2 (CH_2)	1.27 (m), 1.76 (m)	H-30
2	26.8 (CH_2)	1.90 (m), 1.91 (m)		22	28.8 (CH_2)	1.50 (m), 1.99 (m)	
3	88.8 (CH)	3.29 (dd, 5.0, 12.0)	H-23, 24, 1′	23	28.1 (CH_3)	1.27 (s)	H-24
4	39.6 (C)		H-23, 24	24	16.8 (CH_3)	1.10 (s)	H-23, 5
5	56.1 (CH)	0.80 (d, 12.0)	H-23, 24, 25	25	16.2 (CH_3)	0.92 (s)	
6	18.5 (CH_2)	1.27 (d,9.5),1.29 (m)	H-5	26	17.1 (CH_3)	0.84 (s)	
7	34.3 (CH_2)	1.44 (m)	H-26	27	21.5 (CH_3)	1.02 (s)	
8	41.2 (C)		H-26, 27	29	20.8 (CH_3)	1.02 (d, 5.5)	
9	47.6 (CH)	1.56 (dd, 5.5,11.0)	H-25, 26	30	18.9 (CH_3)	0.93 (d, 7.5)	
10	36.9 (C)		H-25	1′	105.9 (CH)	4.81 (d, 7.0)	H-2′
11	23.8 (CH_2)	1.95 (m), 1.98 (m)	H-25	2′	83.0 (CH)	4.20 (m)	H-1″
12	117.5 (CH)	5.64 (*t*-like)	H-11	3′	78.0 (CH)	3.90 (m)	
13	138.2 (C)		H-27	4′	70.9 (CH)	4.13 (m)	
14	39.0 (C)		H-26, 27	5′	66.6 (CH_2)	3.64 (d, 2.0), 4.26 (m)	
15	27.2 (CH_2)	1.99 (m), 2.01 (m)	H-27	1″	105.7 (CH)	5.35 (d, 7.5)	H-3″, 5″
16	29.9 (CH_2)	1.29 (m)		2″	77.0 (CH)	4.08 (t, 8.0)	
17	128.2 (C)			3″	78.3 (CH)	3.91 (m)	
18	130.2 (C)		H-29	4″	71.7 (CH)	4.29 (m)	
19	33.7 (CH)	2.36 (d, 7.5)	H-29, 30	5″	78.0 (CH)	4.20 (m)	
20	33.8 (CH)	2.38 (d, 7.5)	H-29, 30	6″	62.7 (CH_2)	4.42 (dd, 3.0, 11.5)	
						4.47 (dd, 4.5, 12.5)	