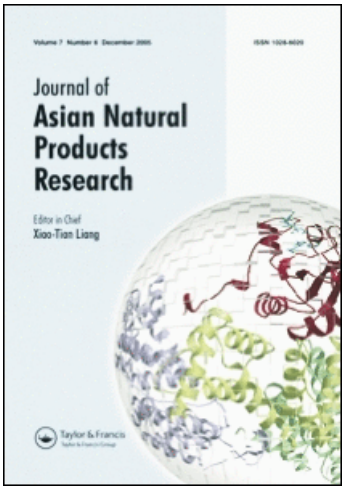

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Three new triterpenoid saponins from the rhizomes of *Impatiens pritzellii* var. *hupehensis*

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Three new triterpenoid saponins, impatiprins A–C (**1–3**), together with a known triterpenoid (**4**) and two known triterpenoid saponins (**5**, **6**), were isolated from the rhizomes of *Impatiens pritzellii* Hook. f. var. *hupehensis* Hook. f. The structures of **1–3** were determined by 1D and 2D NMR, FAB-MS techniques and chemical methods. Compounds **1** and **2** showed weak cytotoxicities against S-180, HeLa and HepG2 cell lines.

Keywords: *Impatiens pritzellii*; Triterpenoid saponins; Impatiprins A–C; Cytotoxicity

1. Introduction

Impatiens is the largest genus of Balsaminaceae with about 200 species in China. *Impatiens* plants are mainly distributed in Southwest China and the Tibet province. *Impatiens pritzellii* Hook. f. var. *hupehensis* Hook. f. grows in the northwest district of Hubei province and Wan county of Chongqing, China. Local people call it 'Lengshuiqi' and use the rhizomes to treat rheumatism, diarrhoea and acute stomach ache [1]. We found that the *n*-BuOH-soluble fraction partitioned from the MeOH extract of the rhizomes of *I. pritzellii* showed anti-inflammatory and analgesic effects [2]. This prompted us to initiate a chemical investigation of the *n*-BuOH-soluble fraction of this plant. This paper reports the isolation and structural determination of three new triterpenoid saponins, impatiprins A–C (**1–3**), along with a known triterpenoid (**4**) and two known triterpenoid saponins (**5** and **6**). Compounds **1** and **2** showed weak cytotoxicities against S-180, HeLa and HepG2 cell lines.

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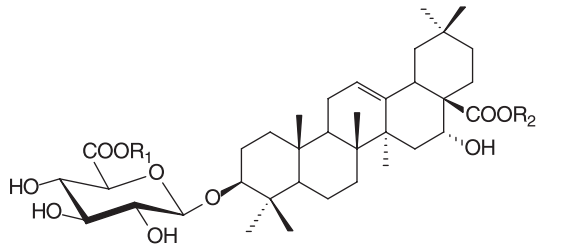
2. Results and discussion

The *n*-BuOH-soluble fraction was chromatographed over silica gel column, followed by repeated Sephadex LH-20 chromatographic purification and preparative HPLC purification, affording six triterpenoid compounds, impatiprins A–C (**1–3**) and three known compounds. The known compounds were isolated from *Impatiens* genus for the first time and identified as 3β,16α-dihydroxyolean-12-eneoic acid (echinocystic acid) (**4**), 3-*O*-β-D-glucuronopyranosyl echinocystic acid (**5**) and 3-*O*-[(6-*O*-methyl)-β-D-glucuronopyranosyl] echinocystic acid (**6**) by comparing their physical and spectroscopic data with those reported in the literature [3].

Impatiprin A (**1**) was obtained as white needles from $CHCl_3$. The negative-ion HRFAB-MS of **1** showed an ion peak at *m/z* 675.4112 $[M - H]^-$, in accordance with an empirical molecular formula of $C_{38}H_{60}O_{10}$, which was supported by its ^{13}C NMR and various DEPT data. Spectroscopic data comparison of **1** with those of **4** and known echinocystic acid glycosides [4–6] suggested that **1** is an echinocystic acid 3-*O*-glycoside. Acid hydrolysis of **1** afforded echinocystic acid and glucuronic acid by TLC and HPLC analysis. Alkaline hydrolysis of **1** afforded **5** identified by TLC and NMR. The anomeric configuration of glucuronopyranose was determined as β on the basis of the large value of the coupling constants ($J = 7.7$ Hz). The chemical shift of C-6 of the glucuronic acid (δ 169.8) in ^{13}C NMR and the following HMBC correlations: H-1 of ester (δ 4.17, 2H, q, $J = 7.1$ Hz) with C-6 of glucuronic acid (δ 169.8), H-2 of ester (δ 1.24, 3H, t, $J = 7.1$ Hz) with C-1 of ester (δ 61.4) showed that the sugar moiety of **1** was 3-*O*-[(6-*O*-ethyl)-β-D-glucuronopyranoside]. So the structure of **1** was established as 3-*O*-[(6-*O*-ethyl)-β-D-glucuronopyranosyl] echinocystic acid, and has been accorded the trivial name, Impatiprin A (figure 1).

Impatiprin B (**2**) was obtained as colourless needles from $CHCl_3$. The negative-ion HRFAB-MS of **2** showed an ion peak at *m/z* 703.4409 $[M - H]^-$, in accordance with an empirical molecular formula of $C_{40}H_{64}O_{10}$. Spectroscopic comparison of **2** and **1** (see tables 1 and 2) showed that the only difference between them can be rationalized to the ester moiety part of 3-*O*-sugar: 6-*O*-ethyl in **1** was replaced by 6-*O*-*n*-butyl in **2**. Acid hydrolysis of **2** also afforded **4** and glucuronic acid. Alkaline hydrolysis of **2** and **1** afforded the same product that was identified as **5** by TLC. After the confirmation of 2D NMR, the structure of **2** was elucidated as 3-*O*-[(6-*O*-*n*-butyl)-β-D-glucuronopyranosyl] echinocystic acid (Impatiprin B) (figure 1). **2** was also obtained from the MeOH extract of the plant, and therefore **2** was a natural product.

Impatiprin C (**3**) was obtained as colourless needles from MeOH. The negative-ion HRFAB-MS of **3** showed an ion peak at *m/z* 1113.2312 $[M - H]^-$, in accordance with an empirical molecular formula of $C_{56}H_{90}O_{22}$. The chemical shifts of δ 91.1 (C-3) and 177.0 (C-28) revealed that **3** was a bisdesmosidic glycoside. Spectroscopic comparison of **3** with **2** (see tables 1 and 2) showed that **3** was different only in 28-*O*-sugar moiety to **2**. Alkaline hydrolysis of **3** also afforded **5**. Acid hydrolysis of **3** afforded **4**, glucuronic acid, xylose and rhamnose identified by TLC and HPLC comparing with the authentic samples. Besides the signals of the sugar linked in C-3, the ^1H NMR and ^{13}C NMR spectra showed three other anomeric proton signals at δ 5.40 (d, $J = 6.7$ Hz), 5.27 (br s) and 4.49 (d, $J = 7.4$ Hz) and the corresponding carbon signals at δ 95.5, 101.1, and 106.9, respectively (see table 2). Starting from the anomeric proton and anomeric carbon signals of each sugar unit, the proton and the carbon signals of the 28-*O*-sugar moiety were assigned by 2D NMR experiments and judged to be two xyloses and one rhamnose. The linkage of the 28-*O*-sugar moiety was established



	R_1	R_2
1	CH_2CH_3	H
2	$CH_2CH_2CH_2CH_3$	H
3	$CH_2CH_2CH_2CH_3$	Xyl I, Xyl II, Rha

Figure 1. Structures of compounds **1**–**3**.

Table 1. ^{13}C NMR data (δ) for aglycone moieties of **1**–**3**[†].

Position	***1***	***2***	***3***
1	39.3	39.3	39.9
2	26.7	26.7	27.1
3	89.8	89.7	91.1
4	40.2	40.2	40.2
5	56.5	56.4	57.1
6	18.9	18.8	19.4
7	32.7	32.7	31.7
8	39.8	39.8	40.8
9	47.6	47.5	48.1
10	37.6	37.6	37.9
11	24.1	24.0	24.5
12	122.9	122.8	123.6
13	144.9	144.8	144.7
14	42.2	42.1	42.7
15	36.3	36.3	36.5
16	74.9	74.9	74.7
17	48.9	48.6	50.2
18	41.5	41.4	42.3
19	47.4	47.3	47.9
20	31.0	31.1	32.0
21	36.1	36.1	36.4
22	33.8	33.8	34.3
23	28.2	28.2	28.5
24	16.8	16.8	17.0
25	15.9	15.8	16.1
26	17.6	17.5	17.8
27	27.2	27.1	27.2
28	178.4	178.5	177.0
29	33.3	33.3	33.3
30	24.7	24.6	24.9

[†] Compounds **1** and **2**: in acetone-d_6; Compound **3**: in CD_3OD.

Table 2. ^{13}C NMR and ^{1}H NMR data for sugar moieties of **1–3**[†].

	1		*2*		*3*	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-*O*-sugar						
Glc A						
1	106.7	4.44 d (7.7)	106.7	4.44 d (7.7)	107.1	4.39 d (7.7)
2	75.1	3.26	75.0	3.26 dd (8.0, 8.8)	75.3	3.22
3	77.4	3.41	77.3	3.41	77.6	3.35
4	72.8	3.58	72.6	3.59	73.1	3.56
5	76.4	3.80 d (9.7)	76.3	3.80 d (9.7)	76.7	3.79 d (9.8)
6	169.8	4.44 d (7.7)	169.7	4.44 d (7.7)	170.9	4.39 d (7.7)
Ester						
1	61.4	4.17 q (7.1)	65.1	4.14	66.2	4.19
2	14.2	1.24 t (7.1)	31.3	2.03	31.3	1.90
3			19.6	1.40	20.1	1.44
4			14.0	0.91 t (7.4)	14.0	0.93 t (7.3)
28-*O*-sugar						
Xyl I						
1					95.5	5.40 d (6.7)
2					76.2	3.60
3					76.7	3.90
4					70.8	3.46
5					66.6	3.90
						3.33
Rha						
1					101.1	5.27 br s
2					71.9	3.85
3					72.3	3.83
4					83.9	3.56
5					68.9	3.70 dq (9.2, 6.2)
6					18.3	1.30 d (6.2)
Xyl II						
1					106.9	4.49 d (7.4)
2					76.1	3.24
3					78.2	3.32
4					71.1	3.46
5					67.3	3.83
						3.22 dd (8.3, 8.6)

[†] Compounds **1** and **2**: in acetone-d_6; Compound **3**: in CD_3OD.

by the following HMBC correlations: H-1 of xylose II (δ 4.49) with C-4 of rhamnose (δ 83.9), H-1 of rhamnose (δ 5.27) with C-2 of xylose I (δ 76.2), H-1 of xylose I (δ 5.40) with C-28 of the aglycone (δ 177.0). The anomeric configurations of both xylopyranoses were determined as β on the basis of the large value of the coupling constants ($J = 6.7, 7.4$ Hz). The anomeric configuration of rhamnopyranose was indicated as α by the ^{13}C NMR chemical shift values of C-3 and C-5 [7]. The spectroscopic data of the 28-*O*-sugar moiety accorded with the corresponding moiety of reported compound [8]. From the above evidence, the structure of **3** was elucidated as 3-*O*-[(6-*O*-*n*-butyl)-β-D-glucuronopyranosyl]-28-*O*-[β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-β-D-xylopyranosyl] echinocystic acid (Impatiprin C) (figure 1).

The three new triterpenoid saponins, impatiprins A–C (**1–3**), were evaluated for their cytotoxic activities against Balb/c mice sarcoma tumour cell (S-180), human cervical squamous carcinoma cell line (HeLa) and human hepatoma cell line (HepG2), contrasting with the clinically used anticancer drug 5-FU (5-fluorouracil). After 72 h treatment, **1** and

Table 3. Cytotoxicities of compounds **1**–**3**.

	IC_{50} (μM)			
Cells	***1***	***2***	***3***	*5-FU*
S-180	106.51	37.40	–	30.92
HeLa	134.57	44.93	–	19.08
HepG2	157.83	55.78	–	48.38

2 exhibited IC_{50} ranges from 37.40 to 157.83 μM, and **3** showed no cytotoxic activity (see table 3).

3. Experimental

3.1 General experimental procedures

Melting points were measured using an XT4–100X micro-melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin–Elmer FT-IR spectrometer. Optical rotation was determined on a Perkin–Elmer digital polarimeter. ^{1}H NMR and ^{13}C NMR data were recorded on a Bruker AM-400 spectrometer at 25°C and TMS was used as internal standard. FAB-MS and HRFAB-MS were conducted using a VG Auto Spec-3000 instrument. Silica gel (100–200 mesh, Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) were used for column chromatography. Preparative HPLC (WellChrom Preparative Pump K-1800, Knauer, Germany) was performed on an ODS column (300 × 20 mm, detector: UV Detector K-2501, Knauer, Germany). Analytic HPLC (Hewlett Packard 1100) was performed by using a Zorbax SB-C18 (5 μm) column (10 × 250 mm) and a UV detector.

3.2 Plant material

The rhizomes of *Impatiens pritzellii* Hook. f. var. *hupehensis* Hook. f. were collected in Enshi area, Hubei province, China, and identified by Professor Dingrong Wan (Hubei Province Institute for Drug Control, China). A voucher specimen (P 20020713) is deposited at the Faculty of Pharmaceutical Sciences, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.

3.3 Extraction and isolation

The finely cut roots of *I. pritzellii* (3.3 kg) were extracted three times with boiling MeOH. The MeOH extract was concentrated at 50°C under reduced pressure to give a viscous residue (1190 g). This residue was suspended in H_2O and partitioned with EtOAc and *n*-BuOH successively, the *n*-BuOH solution was concentrated at 70°C under reduced pressure. The *n*-BuOH-soluble fraction (104.8 g) was chromatographed on silica gel column using $CHCl_3$/MeOH/H_2O gradiently, followed by repeated Sephadex LH-20 chromatographic purification ($CHCl_3$/MeOH 1:1 or pure MeOH) and preparative HPLC purification (70–95% MeOH in Water, UV detector 211 nm), affording **1** (80 mg), **2** (335 mg), **3** (420 mg), **4** (15 mg), **5** (63 mg) and **6** (55 mg), respectively.

3.3.1 Impatiprin A (1). White needles; mp 274–275°C; $[\alpha]_D^{20}$ −13.5 (*c* 0.4, MeOH); IR (KBr) ν_{max} 3437, 2948, 1743, 1696, 1630, 1465, 1387, 1162, 1055 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.29 (1H, br s, H-12), 4.56 (1H, br s, H-16), 3.16 (1H, dd, $J = 11.8$, 4.5 Hz, H-3), 3.05 (1H, dd, $J = 14.0$, 4.1 Hz, H-18), 1.42 (3H, s, H_3-27), 1.05 (3H, s, H_3-23), 0.97 (3H, s, H_3-30), 0.94 (3H, s, H_3-26), 0.87 (3H, s, H_3-29), 0.81 (3H, s, H_3-24), 0.77 (3H, s, H_3-25); Other NMR data: see tables 1 and 2; FAB-MS (positive) *m/z* 677 $[M + H]^+$; HRFAB-MS (negative) *m/z* 675.4112 $[M - H]^-$ (calcd for $C_{38}H_{59}O_{10}$, 675.4108).

3.3.2 Impatiprin B (2). Colourless needles; mp 182–184°C; $[\alpha]_D^{20}$ −13.25 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3436, 2952, 1734, 1700, 1630, 1466, 1388, 1167, 1062 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 5.29 (1H, br s, H-12), 4.55 (1H, br s, H-16), 3.16 (1H, dd, $J = 11.6$, 4.2 Hz, H-3), 3.05 (1H, dd, $J = 14.1$, 4.1 Hz, H-18), 1.43 (3H, s, H_3-27), 1.04 (3H, s, H_3-23), 0.97 (3H, s, H_3-30), 0.93 (3H, s, H_3-26), 0.87 (3H, s, H_3-29), 0.80 (3H, s, H_3-24), 0.76 (3H, s, H_3-25); Other NMR data: see tables 1 and 2; FAB-MS (positive) *m/z* 705 $[M + H]^+$; HRFAB-MS (negative) *m/z* 703.4409 $[M - H]^-$ (calcd for $C_{40}H_{63}O_{10}$, 703.4421).

3.3.3 Impatiprin C (3). Colourless needles; mp 209–211°C; $[\alpha]_D^{20}$ −40.5 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3436, 2952, 1735, 1631, 1000–1200 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 5.31 (1H, br s, H-12), 4.47 (1H, br s, H-16), 3.12 (1H, dd, $J = 11.6$, 4.2 Hz, H-3), 2.95 (1H, dd, $J = 14.1$, 4.1 Hz, H-18), 1.37 (3H, s, H_3-27), 1.05 (3H, s, H_3-23), 0.96 (3H, s, H_3-30), 0.95 (3H, s, H_3-26), 0.87 (3H, s, H_3-29), 0.85 (3H, s, H_3-24), 0.76 (3H, s, H_3-25); Other NMR data: see tables 1 and 2; FAB-MS (positive) *m/z* 1115 $[M + H]^+$; HRFAB-MS (negative) *m/z* 1113.2312 $[M - H]^-$ (calcd for $C_{56}H_{89}O_{22}$, 1113.5846).

3.4 Acid hydrolysis

A solution of saponins (5 mg each) in 1 M HCl (dioxane/H_2O, 1:1, 1 ml) was heated at 100°C in a water bath for 2 h. Echinocystic acid and glucuronic acid were checked out in the reaction mixture by TLC and HPLC comparing with the authentic samples, and two other sugars were also identified in the reaction mixture of **3** as xylose and rhamnose by TLC and HPLC comparing with the authentic samples.

3.5 Alkaline hydrolysis

A solution of saponins (20 mg each) in 2.5% NaOH (MeOH/H_2O, 1:1, 2 ml) was heated at 58°C in a water bath for 1 h. The reaction mixture was adjusted to pH 6 with 3% HCl and then extracted with H_2O-saturated *n*-BuOH (3 × 10 ml). After being purified, prosaponins of **1**–**3** were identified to be the same as **5** by comparing on TLC.

3.6 Cytotoxicity assay

HeLa and HepG2 cell lines, obtained from Tongji Hospital (Wuhan, China), together with S-180, were maintained in DMEM containing 10% foetal bovine serum. Each kind of cell line was washed and resuspended in the above medium to 10^5 cells/ml and 195 μl of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated

in 5% CO_2/air for 24 h at 37°C. After incubation, 5 μl of solution containing the samples was added to give the final concentrations of 4.17, 8.33, 16.67, 33.33, 66.67 μg/ml. The cells were further incubated for 72 h in 5% CO_2/air at 37°C. At the end of incubation, 10 μl of 5 mg/ml MTT was added to every well and the plate was further incubated for 4 h in the above environment. The solution was removed from every well and 200 μl of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed in a microshaker for 10 min and then read on a microplate reader at 550 nm. The cytotoxicity was expressed as IC_{50} value, which reduced the viable cell number by 50%.

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