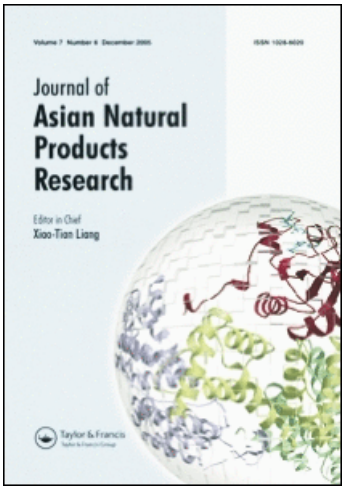

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Phytolacacinoside A, a new triterpenoid saponin from *Phytolacca acinosa* Roxb

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Phytolacacinoside A (**1**), a novel triterpenoid saponin, together with the seven known compounds, was isolated from 75% ethanol extract of the root of *Phytolacca acinosa* Roxb (Phytolaccaceae). Their structures were elucidated on the basis of analysis of spectroscopic data and physicochemical properties as 3-*O*-β-[(β-D-glucopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl)]-11β-methoxy-jaligonic acid 30-methyl ester 28-*O*-β-D-glucopyranoside (**1**), 3-*O*-β-[(β-D-glucopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl)]-jaligonic acid 30-methyl ester 28-*O*-β-D-glucopyranoside (**2**, esculentoside G), 3-*O*-β-[(β-D-glucopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl)]-jaligonic acid 30-methyl ester (**3**, phytolaccoside E), 3-*O*-β-D-xylopyranosyl-jaligonic acid 30-methyl ester (**4**, phytolaccoside B), hypaphorine (**5**), palmitic acid monoglyceride (**6**), β-sitosterol (**7**), and daucosterol (**8**).

Keywords: *Radix phytolaccae*; triterpenoid saponin; Phytolacacinoside A

1. Introduction

Radix phytolaccae, namely Shang Lu in Chinese, has been widely used in Chinese traditional medicine as a diuretic drug for a long history and employed for the treatment of various diseases such as edema, swelling, and sores. It was recorded in Chinese Pharmacopoeia [1] and legally prepared from the roots of *Phytolacca acinosa* Roxb and *Phytolacca americana* L. A number of compounds including triterpenes [2,3], triterpenoid saponins [4–10], lignans [11–14], and cerebrosides [15] have been isolated from the roots and seeds of *P. americana* or *P. acinosa* Roxb as well as triterpene glycosides that have been produced from the cultures of *P. americana* [16]. Most of the saponins exhibited diverse biological activities, including molluscicidal, fungistatic, anti-inflammatory, analgesic, spermicidal, antiduretic, and weak sedative activities. In the recent years, escutentoside A, a main triterpenoid saponin, has been used against tumor, hyperplasia of mammary glands, and endometriosis in clinic and has the potent therapeutic effects. In our phytochemical investigation on the 75% EtOH extract of the roots of *P. acinosa* Roxb, phytolacacinoside A (**1**), a new triterpenoid saponin, was isolated together with seven known compounds esculentoside G (**2**) [5,16–18], phytolaccoside E (**3**) [6,16], phytolaccoside B (**4**) [6], hypaphorine (**5**) [19], palmitic acid monoglyceride (**6**), β-sitosterol (**7**), and daucosterol (**8**). Among these constituents mentioned, compounds **5** and **6** are reported from *P. acinosa* Roxb for the first time. In the present paper, we had briefly described the

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isolation of phytolacacinoside A (**1**) and its structure elucidation.

2. Results and discussion

The dried roots of *P. acinosa* (5.0 kg) were extracted with 75% ethanol and the concentrated extract was fractionated on HPD_{100} macroporous resin column chromatography to afford five subfractions. The subfraction was isolated and then purified by a combination of silica gel, ODS (Fuji Silysia Chemical, Japan), and Sephadex LH-20 (Mitsubishi Chemical, Japan) column chromatography, to yield compounds **1**–**8**.

The molecular formula of **1** was established as $C_{49}H_{78}O_{22}$ by positive HR-ESI-MS (*m/z* 1057.4606 $[M+K]^+$) and negative ESI-MS (*m/z* 1053 $[M+Cl]^-$ and 891 $[M+Cl\text{-}162]^-$) experiments. The ^1H and ^{13}C NMR spectral data of **1** indicated the presence of a sapogenin 2-hydroxy acinosolic acid 30-methyl ester and oligosaccharide moieties. ^1H NMR spectrum exhibited three anomeric protons at δ 6.33 (1H, d, $J = 8.0$ Hz), 5.04 (1H, d, $J = 8.0$ Hz), and 4.95 (1H, d, $J = 8.0$ Hz), an olefinic proton at δ 5.83 (1H, d, $J = 4.4$ Hz), as well as five tertiary methyl signals at δ 1.72 (3H, s), 1.36 (3H, s), 1.30 (3H, s), 1.18 (3H, s), and 1.14 (3H, s). ^{13}C NMR spectrum displayed three anomeric carbons at δ 106.4, 103.6, and 95.8, a pair of olefinic carbon at δ 123.5 and 148.1, along with an ester carbonyl carbon at δ 176.1. In addition, acid hydrolysis of **1** confirmed the sugar moiety composed of glucose and xylose which were identified by co-chromatographic examinations on thin layer plates with authentic samples. From the above spectroscopic and chemical information, compound **1** was deduced to be a bisdesmosidic triterpenoid saponin with two glucopyranosyl units and one xylopyranosyl unit, disubstituted at the positions C-3 and C-28. A detailed comparison of ^{13}C NMR spectrum of **1** with those of the reported esculentoside G (**2**) indicated that the data of sugar moieties and major positions of aglycone of **1** were in accordance with those of **2**, except for the obvious differences of chemical shifts in C-8, C-9, C-10, C-11, C-13, C-25, and C-26 (see Table 1). Further information was observed that the chemical shift of C-11 was at δ 76.2 and an additional methoxyl group at δ 54.7 was present in **1**. The above evidences implied that **1** was an oxygenated derivative of **2** substituted by the methoxy group at the positions C-11, which was ascertained by the key HMBC correlations between H-9 at δ 1.98 (1H, d, $J = 4.0$ Hz), H-12 at δ 5.83 (1H, d, $J = 4.0$ Hz), 11-OCH_3 at δ 3.20, and C-11 at δ 76.2, respectively. The other proton and carbon signals of **1** were also assigned by the aid of 2D NMR experiments such as COSY, DEPT, HMQC, and HMBC and by the comparison with the data of **2** in the literature (see Table 2). Accordingly, the structure of **1** was preliminarily established as 3-*O*-β-[(β-D-glucopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl)]-11-methoxy-jaligonic acid 30-methyl ester 28-*O*-β-D-glucopyranoside.

In addition, the relative configuration of 11-OCH_3 was determined by the following evidences. From the proposed structure of **1**, no proton would be spin-coupled with H-9 but H-11, meanwhile, the coupling constant of H-9 was found to be 4.0 Hz, namely, a–e spin-coupling is present between H-9 and H-11. The α-configuration of H-11 was determined due to the α-configuration of H-9. The proposed stereostructure of **1** was further simulated by Alchemy III Software. If α-configuration of methoxyl group was located at C-11, $\theta_{9\alpha H,11\beta H}$ was measured as 161.2°, the coupling constant ($J_{9\alpha H,11\beta H}$) was calculated as ~8.0 Hz, which was in accordance with the reported data of Ilwensisaponin C, a saponin with 11α-OCH_3 isolated from *Verbascum pterocalycinum* var. *mutense* [20]. When 11-OCH_3 was simulated as β-configuration, a–e spin-coupling of H-9 and H-11 was obviously observed. According to the information mentioned, 11-OCH_3 was

Table 1. ^{13}C NMR spectral data for compounds **1**–**4** in pyridine-d_5.

No.	**1**	**2**	**3**	**4**	Sugar	**1**	**2**	**3**	**4**
1	45.1	44.1	43.8	43.6	3-*O*-xyl-1	106.4	106.3	106.0	106.8
2	70.9	70.7	70.6	71.0	2	75.0	74.9	74.8	75.4
3	82.7	82.8	82.7	82.9	3	76.4	76.2	76.0	78.5
4	42.9	42.2	42.0	42.8	4	77.4	78.7	77.5	71.1
5	47.8	48.4	47.3	48.6	5	65.2	65.0	64.3	67.2
6	18.2	17.9	17.7	18.0	glc′-1	103.6	103.4	103.2	
7	33.3	32.7	32.7	33.0	2	74.2	74.1	74.2	
8	43.4	39.9	39.6	39.8	3	78.9	78.7	77.9	
9	53.9	47.5	48.3	47.7	4	71.7	71.6	71.5	
10	38.4	36.8	36.7	37.0	5	78.1	77.4	78.5	
11	76.2	23.8	23.6	24.0	6	62.6	62.5	62.4	
12	123.5	123.5	123.5	123.5	28-*O*-glc-1	95.8	95.7		
13	148.1	143.7	144.3	144.0	2	74.2	74.0		
14	42.1	43.1	42.5	42.2	3	79.4	79.2		
15	28.4	28.2	28.0	28.4	4	71.2	70.7		
16	23.4	23.4	23.7	23.9	5	78.9	77.4		
17	46.1	46.4	46.0	46.3	6	61.9	61.7		
18	42.6	43.9	43.2	43.0					
19	42.3	42.7	42.4	42.8					
20	43.9	43.9	44.0	44.2					
21	30.5	30.4	30.5	31.0					
22	33.9	33.9	34.3	34.8					
23	64.7	64.5	64.7	65.3					
24	14.9	14.9	14.7	15.0					
25	19.0	17.5	17.0	17.6					
26	19.3	17.2	17.3	17.3					
27	25.4	26.0	26.0	26.2					
28	176.8	176.9	179.8	179.0					
29	28.2	28.2	28.2	28.6					
30	176.1	176.0	177.2	177.3					
$COOCH_3$	51.7	51.6	51.6	51.6					
11-OCH_3	54.7								

indicated as β-configuration. The structure of **1** was finally identified as 3-*O*-β-[(β-D-glucopyranosyl-(1 → 4)-*O*-β-D-xylopyranosyl)]-11β-methoxy-jaligonic acid 30-methyl ester 28-*O*-β-D-glucopyranoside, named phytolacacinoside A.

3. Experimental

3.1 General experimental procedures

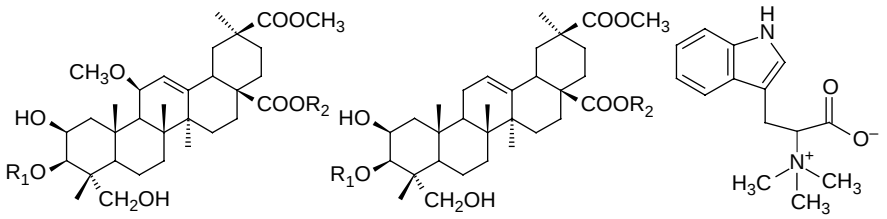
NMR spectra were recorded at 400 and 100 MHz for ^1H and ^{13}C, respectively, on a JEOL Teol 400 MHz NMR spectrometer in pyridine-d_5 with TMS as internal standard. ESI-MS or HR-ESI-MS were recorded on a Jabstec (Micromass, Manchester, England) mass spectrometer. Column chromatography was performed with silica gel (200–300 mesh; Qingdao Haiyang Chemical Co., Qingdao, China) and Sephadex LH-20. TLC was carried out with precoated silica gel plates (GF-254; Qingdao Haiyang Chemical Co.). The reference glucose and xylose were bought from Sigma Company (Poole, Dorset, UK). All solvents were of analytical grade.

3.2 Plant material

The dried root of *P. acinosa* was collected in Beijing, China, in May 2006 and was identified by Associate Professor Xirong HE (Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China) as the roots of *P. acinosa* Roxb. The voucher specimens

Table 2. ^{1}H NMR spectroscopic data and the key HMBC correlations of compound **1**.

No.	δ_H	HMBC	No.	δ_H	HMBC
1	2.64 (1H, br d, $J = 13.0$), 1.76 (1H, br d, $J = 14.0$)	C-2, C-3	3-xyl-1	4.95 (1H, d, $J = 8.0$)	aglycone-C-3
2	4.38		2	3.93 (1H, t, $J = 8.0$)	
3	4.24	C-2	3	4.08 (1H, d, $J = 9.0$)	
5	1.87	C-4, C-10, C-24, C-25	4	4.24	
6	1.58	C-5	5	3.66 (1H, d, $J = 9.0$), 4.34 (1H, dd, $J = 4.0$, 11.5)	
9	1.98 (1H, d, $J = 4.0$)	C-1, C-8, C-10, C-11, C-25, C-26	3-glc-1	5.04 (1H, d, $J = 8.0$)	xyl-C-4
11	3.98 (1H, overlapped)		2	4.03 (1H, t, $J = 7.0$)	
12	5.83 (1H, d, $J = 4.0$)	C-9, C-11	3	4.28 (1H, t, $J = 9.0$)	
18	3.31 (1H, dd, $J = 10.0$)		4	4.18	
19	2.31 (1H, d, $J = 14.0$), 1.72 (1H, d, $J = 14.0$)		5	4.20	
21	2.06		6	4.55 (1H, d, $J = 8.0$), 4.34	
22	1.91		28-glc-1	6.33 (1H, d, $J = 8.0$)	aglycone-C-28
23	3.56 (1H, d, $J = 11.0$), 4.34		2	4.03	
24	1.36 (3H, s)	C-3, C-4, C-5, C-23	3	4.04	
25	1.72 (3H, s)	C-1, C-5, C-9, C-10	4	4.72 (1H, br s)	
26	1.18 (3H, s)	C-7, C-9, C-14	5	4.20	
27	1.30 (3H, s)	C-15	6	4.38	
29	1.14 (3H, s)	C-21			
$COOCH_3$	3.59 (3H, s)				
11-OCH_3	3.20 (3H, s)	C-11			



1 R_1 = glc(1→4)xyl, R_2 = glc

2 R_1 = glc(1→4)xyl, R_2 = glc
3 R_1 = glc(1→4)xyl, R_2 = H
4 R_1 = xyl, R_2 = H

5

Figure 1. Structures of compounds **1**–**5**.

(No. SL-200605) have been deposited in our laboratory.

3.3 Extraction and isolation

The dried roots of *P. acinosa* (5.0 kg) were extracted twice with 75% ethanol by refluxing. The extract was combined and concentrated under reduced pressure to give a brown syrupy extract. The concentrated extract was suspended in water and fractioned by HPD_{100} macroporous resin column chromatography, which was eluted with H_2O and EtOH–H_2O (3:7, 5:5, 7:3, 95:5) to afford five fractions I–V, respectively. The fraction II (2.5 g) was subjected to silica gel column chromatography ($CHCl_3$/MeOH/H_2O 65:35:10 under layer) and purified on ODS column chromatography (MeOH/H_2O 1:1) to afford **5** (19 mg). The fraction III (35 g) was isolated on silica gel column chromatography ($CHCl_3$/MeOH/H_2O 40:10:1) and purified by ODS column chromatography (MeOH/H_2O 6:4) and subjected to recrystallization to yield **1** (11 mg), **2** (164 mg), and **3** (1500 mg). The fraction IV (11 g) was subjected to silica gel column chromatography and purified by ODS and Sephadex LH-20 column chromatography to give **4** (13 mg) and **8** (23 mg). The fraction V (8.0 g) was subjected to vacuum liquid chromatography (petroleum ether/acetone 9:1–6:4) to give **6** (5 mg) and **7** (37 mg).

3.3.1 Phytolacacinoside A (*1*)

White amorphous powder; ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. ESI-MS (negative): *m/z* 1053 $[M+Cl]^-$, 891 $[M+Cl\text{-}162]^-$. HR-ESI-MS (positive): *m/z* 1057.4606 $[M+K]^+$ (calcd for $C_{49}H_{78}O_{22}K$, 1057.4616), 1041.4872 $[M+Na]^+$ (calcd for $C_{49}H_{78}O_{22}Na$, 1041.4877) (Figure 1).

3.4 Acid hydrolysis of phytolacacinoside A (*1*)

A solution of **1** in 2.0 M trifluoroacetic acid (2 ml) was sealed in a tube and heated at 110°C for 6 h. The reaction mixture was extracted with EtOAc. The H_2O layer was concentrated under reduced pressure for the identification of D-glucose and xylose by comparison of the R_f value with that of the authentic sample on TLC.

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