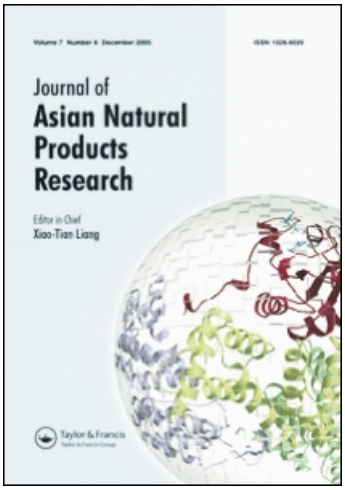

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TWO NEW TRITERPENOID SAPONINS FROM *ADINA PILULIFERA*

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Two new triterpenoid saponins, 3β-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl-pyrocincholic acid 28-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl ester (**1**), and 3β-*O*-β-D-xylopyranosyl(1 → 3)-α-L-rhamnopyranosyl-cincholic acid 28-*O*-β-D-glucopyranosyl ester (**2**) were isolated from the roots of *Adina pilulifera*, together with 18 known compounds. The structures of **1** and **2** were determined by spectroscopic methods.

Keywords: *Adina pilulifera*; Rubiaceae; Triterpenoid saponins

INTRODUCTION

The roots of *Adina pilulifera* (Lam.) Franch. ex Drake (Rubiaceae) are used in traditional Chinese medicine to treat dysentery, enteritis, dropsy, eczema and ulcers [1]. To our knowledge, no phytochemical investigation on the roots of this plant has been reported. In the present study, the 70% aqueous EtOH extract of the roots of *A. pilulifera* was separated by repeated column chromatography to give 20 compounds, namely, 3β-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl-pyrocincholic acid 28-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl ester (**1**), 3β-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl-cincholic acid 28-*O*-β-D-glucopyranosyl ester (**2**), quinovic acid (**3**) [2], 3-oxours-12-ene-27,28-dioic acid (**4**) [2], noreugenin (**5**) [2], 3β-*O*-β-D-fucopyranosyl-quinovic acid (**6**) [3], 3β-*O*-β-D-quinovopyranosyl-quinovic acid (**7**) [3], 3β-*O*-α-L-rhamnopyranosyl-quinovic acid (**8**) [2], 3β-*O*-β-D-glucopyranosyl-quinovic acid (**9**) [2], quinovic acid (28 → 1)-*O*-β-D-glucopyranoside (**10**) [4], 3β-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl-quinovic acid (**11**) [5], 3β-*O*-β-D-glucopyranosyl-quinovic acid 28-*O*-β-D-glucopyranosyl ester (**12**) [6], loganin (**13**) [7], 3β-*O*-β-D-fucopyranosyl-quinovic acid 28-*O*-β-D-glucopyranosyl ester (**14**) [8], 3β-*O*-β-D-quinovopyranosyl-quinovic acid 28-*O*-β-D-glucopyranosyl ester (**15**) [9], 3β-*O*-β-D-xylopyranosyl(1 → 3)-α-L-rhamnopyranosyl-quinovic acid 28-*O*-β-D-glucopyranosyl ester (**16**) [5], 3β-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-quinovic acid 28-*O*-β-D-glucopyranosyl ester (**17**) [10], 7α-morroniside (**18**) [11], 7β-morroniside (**19**) [11] and 3α,5α-tetrahydrodeoxycordifoline (**20**) [12]. Compounds **1** and **2** are new

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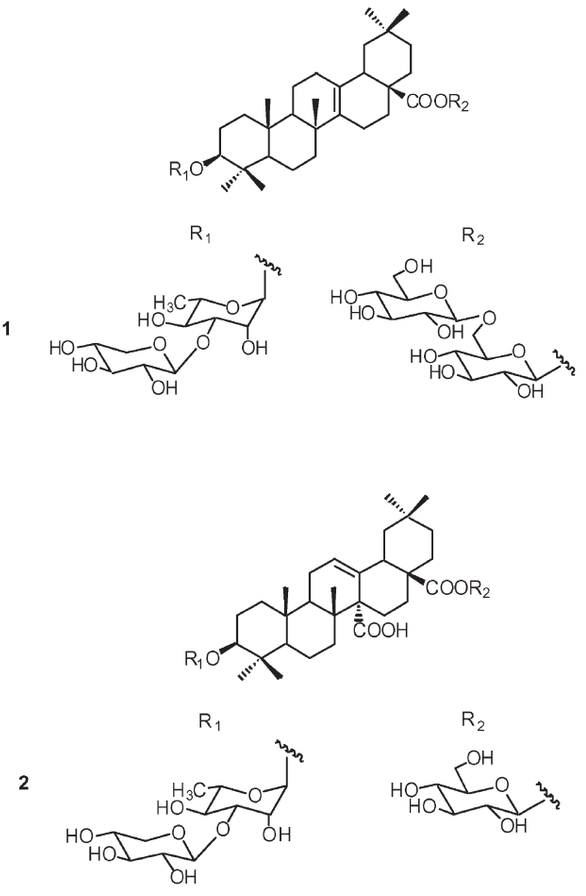
triterpenoid saponins. Compounds **3**–**20** were isolated from this plant for the first time. In this paper, the isolation and structural elucidation of **1** and **2** are described in detail.



RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder, whose molecular formula, $C_{52}H_{84}O_{21}$, was deduced from HRESIMS ($[M + Na]^+$ *m/z* 1067.5398), and it was supported by ^{13}C NMR and DEPT spectra. The ^{13}C NMR spectrum of the aglycon showed 29 carbon signals, including 2 quaternary olefinic carbon signals (δ 130.5 and 137.0) and one carboxyl carbon (δ 176.8). No olefinic proton signal was observed in the ^1H NMR spectrum. Comparison of the ^{13}C NMR data of **1** with those of 3β-*O*-α-L-rhamnopyranosyl-pyrocincholic acid 28-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl ester [13], revealed that the carbon signals of the aglycon of the two compounds were almost identical, indicating that compound **1** was a bisdesmoside of pyrocincholic acid, with glycosidation sites at C-3 (δ 88.8) and C-28 (δ 176.8). The ^1H NMR spectrum of **1** displayed signals of four anomeric protons at δ 6.30 (d, $J = 7.9$ Hz), 5.40 (d, $J = 7.5$ Hz), 5.38 (brs), and 5.09 (d, $J = 7.7$ Hz), which correlated with the carbon signals at δ 95.8, 107.4, 104.5 and 105.4, respectively, in the HMQC

spectrum. Acid hydrolysis of **1** afforded a mixture of glucose, xylose and rhamnose, which were identified by comparison with authentic samples by HPTLC. In the HMBC spectrum of **1**, correlation signals were observed between H-1 (δ 5.38) of rhamnose and C-3 (δ 88.8) of the aglycon, and between H-1 (δ 6.30) of the inner glucose and C-28 (δ 176.8) of the aglycon. This evidence suggested that the rhamnose and inner glucose are attached to the C-3 and C-28 positions, respectively. The linkage of the sugar units at C-3 was established from the HMBC correlation of H-1 (δ 5.40) of xylose with C-3 (δ 83.4) of rhamnose. A cross peak due to the long-range correlation between H-1 (δ 5.09) of the terminal glucose with C-6 (δ 69.7) of the inner glucose, indicated that terminal glucose was the second unit of the disaccharide chain at C-28 of the aglycon. These glycosidic linkages were confirmed by NOESY correlation between H-3 (δ 3.28) of the aglycon and H-1 (δ 5.38) of rhamnose, H-1 (δ 5.40) of xylose and H-3 (δ 4.58) of rhamnose, H-1 (δ 5.09) of terminal glucose and H-6 (δ 4.79 and 4.76) of inner glucose. Full assignments of the ^{1}H and ^{13}C NMR signals were secured from the ^{1}H–^{1}H COSY, HMQC, HMBC, NOESY and TOCSY spectra (Table I). On the basis of the above results, **1** was determined to be 3β-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl-pyrocincholic acid 28-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranosyl ester.

Compound **2** was obtained as an amorphous powder, whose molecular formula, $C_{47}H_{74}O_{18}$, was deduced from HRESIMS ($[M + Na]^{+}$ *m/z* 949.4769), and it was supported by ^{13}C NMR and DEPT spectra. The ^{13}C NMR spectrum of the aglycon showed 30 carbon signals, including 2 olefinic carbon signals (δ 126.2 and 137.9) and two carboxyl carbons

TABLE I ^{1}H and ^{13}C NMR spectral data in pyridine-d_5 at 400/100 MHz for compounds **1** and **2**

	1		**2**			**1**		**2**	
C	δ_H	δ_C	δ_H	δ_C	*C*	δ_H	δ_C	δ_H	δ_C
1	1.74,0.92	38.4	1.67,1.15	39.0		Rha(1 → C3)		Rha(1 → C3)	
2	2.07,1.85	26.2	1.97,1.80	26.1	1′	5.38	104.5	5.22	104.2
3	3.28	88.8	3.07	88.3	2′	4.82	72.1	4.74	72.0
4		39.4		39.2	3′	4.58	83.4	4.56	83.4
5	0.78	55.6	0.91	55.7	4′	4.52	73.2	4.47	73.1
6	1.53,1.39	18.9	1.47,1.33	18.8	5′	4.42	69.9	4.38	69.8
7	1.87,1.19	39.7	1.88,1.81	37.4	6′	1.71	18.5	1.67	18.5
8		38.1		40.1		Xyl(1 → 3rha)		Xyl(1 → 3rha)	
9	1.10	56.6	2.74	47.6	1″	5.40	107.4	5.35	107.3
10		37.3		37.2	2″	4.13	75.8	4.11	75.7
11	1.71,1.65	18.2	2.17	23.7	3″	4.20	78.5	4.18	78.4
12	2.38,2.11	32.2	6.09	126.2	4″	4.38	71.2	4.23	71.2
13		130.5		137.9	5″	4.16,3.73	67.5	4.34,3.70	67.4
14		137.0		57.0		Glc(1 → C28)		Glc(1 → C28)	
15	2.71,2.22	21.1	2.67,2.41	25.5	1‴	6.30	95.8	6.46	95.8
16	2.10	24.2	2.63,2.26	25.1	2‴	4.19	74.2	4.28	74.4
17		45.9		48.2	3‴	4.28	78.9	4.37	79.0
18	2.84	39.6	3.43	44.2	4‴	4.25	71.2	4.42	71.4
19	1.73,1.27	41.6	1.94,1.57	44.0	5‴	4.16	78.0	4.12	79.4
20		30.7		30.9	6‴	4.79,4.76	69.7	4.54,4.49	62.6
21	1.43,1.25	34.5	1.40,1.10	34.1		Glc(1 → 6glc)			
22	2.07,1.82	31.4	1.98,1.80	32.4	1⁗	5.09	105.4		
23	1.00	28.3	0.83	28.3	2⁗	4.07	75.3		
24	0.89	16.6	0.87	17.0	3⁗	4.26	78.5		
25	0.91	16.8	1.01	16.6	4⁗	4.25	71.8		
26	1.22	21.0	1.30	19.1	5⁗	3.96	78.5		
27				178.8	6⁗	4.55,4.43	62.9		
28		176.8		176.8					
29	0.99	32.5	0.83	33.2					
30	0.99	25.0	0.96	23.9					

(δ 176.8 and 178.8). One trisubstituted olefinic proton (δ 6.09, brs) was observed in the ^{1}H NMR spectrum. A comparison between the ^{13}C NMR spectra of **2** and 3β-*O*-β-D-glucopyranosyl-cincholic acid 28-*O*-β-D-glucopyranosyl ester [14] revealed that the carbon signals of the aglycons were almost identical, indicating that the aglycon of compound **2** was cincholic acid, and the glycosidation sites were C-3 and C-28. In the HMQC spectrum, three anomeric carbons at δ 95.8, 107.3 and 104.2 gave correlation with three anomeric proton signals at δ 6.46 (d, $J = 7.9$ Hz), 5.35 (d, $J = 7.4$ Hz), 5.22 (brs), respectively. Upon acid hydrolysis of **2**, glucose, xylose and rhamnose were identified by HPTLC. The HMBC spectrum of **2** displayed correlations between H-1 (δ 5.22) of rhamnose and C-3 (δ 88.3) of the aglycon, H-1 (δ 5.35) of xylose and C-3 (δ 83.4) of rhamnose, H-1 (δ 6.46) of glucose and C-28 (δ 176.8) of the aglycon, indicating the presence of 3-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl and 28-*O*-β-D-glucopyranosyl linkages. In addition, a comparison of the ^{13}C NMR spectra of **2** and **16** [5] revealed that the carbon signals of the sugar moiety of the two compounds were almost identical, indicating that the two compounds have the same sugar moiety, which further confirmed the sugar linkages of **2**. Full assignments of the ^{1}H and ^{13}C NMR signals were secured from the ^{1}H–^{1}H COSY, HMQC, HMBC, NOESY and TOCSY spectra (Table I). On the basis of these results, **2** was determined to be 3β-*O*-β-D-xylopyranosyl (1 → 3)-α-L-rhamnopyranosyl-cincholic acid 28-*O*-β-D-glucopyranosyl ester.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were measured on a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR. ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D NMR spectra were recorded on Bruker DRX-400 and JEOL JNM-EX 400 spectrometers. HRESIMS were obtained on a Bruker APEX FT-MS instrument. Column chromatography was performed on Si gel (Marine Chemical Factory, Qindao, China), Sephadex LH-20 (Pharmacia), octadecyl Si gel (ODS) (10–40 μm, Merck). TLC was conducted on Si gel 60 F_{254} and RP-18 F_{254} plates (Merck). Preparative HPLC was performed using a Waters μBondapak C_{18}, 19 × 300 mm, 10 μm column.

Plant Material

The roots of *Adina pilulifera* (Lam.) Franch. ex Drake were collected from Fujian Province, China in October 2000, and were identified by Dr Minjian Qin. A voucher specimen (No.001012) was deposited in the herbarium of the China Pharmaceutical University.

Extraction and Isolation

Dried roots of *A. pilulifera* (5.0 kg) were extracted three times with 70% EtOH for 2 h each time, with the solvent removed under reduced pressure. The 70% ethanolic extract was suspended in water, then was partitioned with EtOAc and *n*-BuOH, successively. The EtOAc-soluble fraction was concentrated and subjected to Si gel column chromatography eluting with $CHCl_3$–MeOH (1 : 0 → 1 : 1) to yield eight fractions. Fraction 4 was subjected to Si gel ($CHCl_3$–MeOH, 10:1) and Sephadex LH-20 (MeOH) chromatography to give **3** (1.5 g), **4** (30 mg), and **5** (1.2 g). Fraction 6 was subjected to Si gel ($CHCl_3$–MeOH, 10:1) and ODS (MeOH–H_2O, 3:2) chromatography to give a mixture of **6**, **7** and **8**, which were

separated by preparative HPLC [MeOH–H_2O–TFA (65:35:0.1), flow rate = 10 ml/min, detection at 208 nm] to furnish **6** (30 mg), **7** (25 mg), and **8** (35 mg). Fraction 7 was subjected to Si gel ($CHCl_3$–MeOH, 10:1) and ODS (MeOH–H_2O, 3:2) chromatography to give **9** (80 mg), and **10** (50 mg). The *n*-BuOH soluble fraction was concentrated and subjected to Si gel chromatography eluting with $CHCl_3$–MeOH (20 : 1 → 1 : 1) to yield ten fractions. Fraction 2 was subjected to Si gel chromatography eluting with $CHCl_3$–MeOH (8:1) to give **11** (50 mg), and **12** (1.2 g). Fraction 3 was subjected to Si gel ($CHCl_3$–MeOH, 6:1) and ODS (MeOH–H_2O, 3:2) chromatography to give **13** (1.5 g) and a mixture of **14** and **15**, which was separated by preparative HPLC [CH_3CN–H_2O–TFA (32:68:0.1), flow rate = 10 ml/min, detection at 208 nm] to furnish **14** (20 mg), and **15** (22 mg). Fraction 5 was subjected to Si gel chromatography eluting with $CHCl_3$–MeOH (8:1) to give a mixture of **16** and **2**, which was separated by preparative HPLC [CH_3CN–H_2O–TFA (30:70:0.1), flow rate = 10 ml/min, detection at 208 nm] to furnish **16** (60 mg), and **2** (18 mg). Fraction 7 was subjected to Si gel ($CHCl_3$–MeOH, 7:3) and ODS (MeOH–H_2O, 3 : 7 → 7 : 3) chromatography to give **17** (40 mg), a mixture of **18** and **19** (150 mg), **20** (80 mg), **1** (50 mg).

Acid Hydrolysis of 1 and 2

A solution of each saponin (3 mg) in 10% HCl (3 ml) was refluxed on a water bath for 4 h. The solution was diluted with H_2O, neutralized with Ag_2CO_3, and the precipitates were removed by filtration. The filtrate revealed the presence of glucose, xylose and rhamnose by HPTLC ($CHCl_3$–CH_3OH–H_2O, 6.5:3.5:1, lower layer) when compared with authentic samples.

Compound 1: amorphous white powder; $[\alpha]_D^{25}$ − 48.7 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3418 (br), 2941, 1732, 1387, 1055 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table I; HRESIMS *m/z* 1067.5398 $[M + Na]^+$ (calcd for $C_{52}H_{84}O_{21}Na$ 1067.5406).

Compound 2: amorphous white powder; $[\alpha]_D^{25}$ + 16.6 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3421 (br), 2936, 1734, 1388, 1055 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table I; HRESIMS *m/z* 949.4769 $[M + Na]^+$ (calcd for $C_{47}H_{74}O_{18}Na$ 949.4776).

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