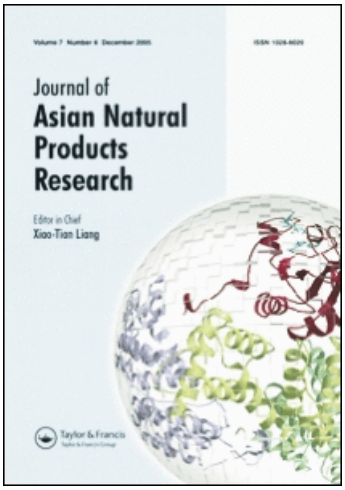

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A NEW ISOMER OF JULIBROSIDE J_2 FROM *ALBIZIA JULIBRISSIN*

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A new isomer of Julibroside J_2 (Chen, S.P., Zhang, R.Y., Ma, L.B. and Tu, G.Z. *Acta Pharm. Sinica*, 1997, **32**, 110–115) was obtained from the cytotoxic fraction of 95% ethanol extracts of stem barks of *Albizia julibrissin* Durazz, together with Julibroside J_2. Its structure was elucidated as 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl]-21-*O*-{(6S)-2-trans-2-hydroxymethyl-6-methyl-6-*O*-[3-*O*-((6S)-2-trans-2-hydroxymethyl-6-methyl-6-hydroxy-2,7-octadienoyl)-β-D-quinovopyranosyl]-2,7-octadienoyl} acacic acid 28-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester(**1**), named as Julibroside J_7, based on chemical and spectral methods. Julibroside J_2 showed good inhibitory action against KB cell line *in vitro*.

Keywords: *Albizia julibrissin*; Leguminosae; Triterpenoid saponins; Cytotoxicity; Julibroside J_7

INTRODUCTION

Albizia julibrissin is a leguminous plant which grows almost all over the world. In China, it is usually planted as an ornamental plant. Its stem barks have been used as sedative agents for hundreds of years according to ancient medical books of China. As a traditional Chinese medicine, its Chinese name is "He Huan Pi", and it is specified in *Chinese Pharmacopoeia* (1995 edition) as a sedative [2] and anti-inflammatory drug to treat injuries from falls and remove carbuncles.

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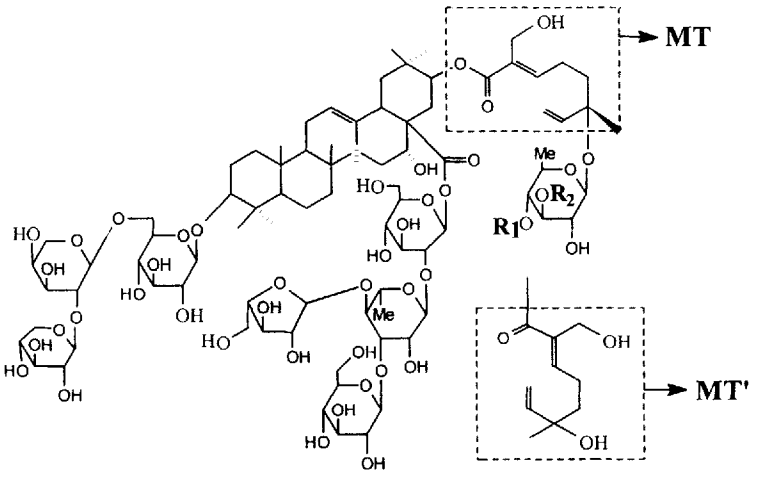

	R_1	R_2
Julibroside J_7(**1**)	H	MT'
Julibroside J_2(**2**)	MT'	H

FIGURE 1 Structures of Julibrosides J_2 and J_7.

In our previous research [1,3], we isolated three novel and complex triterpenoid saponins (Julibrosides J_1, J_2 and J_3). In the present paper, we report the isolation and structure elucidation of a pair of isomers (**1** and **2**, Fig. 1). Compound **2** showed good inhibitory action against KB cell line *in vitro*.

RESULTS AND DISCUSSION

Ninety-five percent ethanol extracts of stem barks of *A. julibrissin* were partitioned between H_2O and $CHCl_3$, EtOAc, n-BuOH, respectively. The n-BuOH-soluble part was chromatographed over D_{101} macroporous resin columns, Sephadex LH-20 columns and silica gel columns to afford colorless powders (Frs 41–43). A pair of isomers (**1** and **2**) were obtained from the merged fraction by means of repeated Rp C_{18} column chromatography and preparative HPLC.

Saponin **1** was obtained as a white powder. It showed positive Molish and Liebermann–Buchard reactions, which suggested a saponin skeleton for **1**. When **1** was hydrolyzed with refluxing 2.0 mol/L HCl, its sapogenin was found to be identical to the authentic sample: acacic acid lactone on HPTLC in the hydrolysate. D-glucose, L-arabinose, D-xylose, L-rhamnose and D-quinovose were found to be present in the hydrolysate also, compared with authentic samples (with the literature [4] for D-quinovose). After recording ^{1}H- and ^{13}C NMR spectra for **1**, the resonances were assigned on the basis of extensive 2D ^{1}H–^{1}H homonuclear, and 2D ^{1}H–^{13}C heteronuclear NMR experiments. In the ^{1}H NMR spectrum of **1**, seven three-proton singlets at δ 0.96, 1.01, 1.05, 1.09, 1.15, 1.29, 1.86, three multiplets at δ 3.54, 5.21, 6.29, and a broad singlet at δ 5.62 were attributed to the presence of seven tertiary methyl and three secondary hydroxyl groups at C-3, C-16, C-21, and an olefinic proton at C-12 of the sapogenin moiety, respectively. The ^{13}C NMR spectrum of **1** showed 30 distinct resonances assigned to aglycone (Table I). The spectral data for aglycone moiety of **1** were in good agreement with those [5] published for substituted acacic acid, from which the acacic acid was confirmed further to be the aglycone of **1**.

In the ^{1}H NMR spectrum of **1**, eight resonances for the anomeric protons of the sugar moieties were observed at δ 4.84 (1H, d, J = 7.8 Hz, H-qui-1), 4.87 (1H, d, J = 7.8 Hz, H-glc-1), 4.99 (1H, d, J = 6.6 Hz, H-xyl-1), 5.14 (1H, br s, H-arap-1), 5.30 (1H, d, J = 7.6 Hz, H-glc″-1), 5.88 (1H, br s, H-rha-1), 6.02 (1H, d, J = 8.0 Hz, H-glc′-1) and 6.23 (1H, br s, H-araf-1). Two three-proton doublets at δ 1.57 (J = 5.7 Hz) and 1.74 (J = 5.8 Hz) due to methyls of deoxy-sugar moieties: quinovose and rhamnose, were also

TABLE I ^{13}C NMR data for aglycone moieties of J_1, **1** and **2** (pyridine-d_5)

C	J_1	**1**	**2**	C	J_1	**1**	**2**
1	38.9	39.0	39.2	16	74.8	73.9	73.9
2	26.9	26.6	26.9	17	51.7	51.6	51.8
3	88.9	88.8	88.4	18	40.9	40.8	40.9
4	39.7	39.6	39.7	19	47.9	47.8	48.1
5	56.1	56.0	56.1	20	35.6	35.5	35.5
6	18.7	18.8	18.4	21	77.1	76.8	76.9
7	33.7	33.6	33.8	22	36.4	36.4	36.5
8	40.2	40.1	40.3	23	28.3	28.2	28.4
9	47.2	47.1	47.3	24	17.1	17.1	17.1
10	37.1	37.1	37.3	25	15.9	15.9	15.9
11	23.9	23.7	23.9	26	17.3	17.3	17.5
12	124.1	123.1	123.1	27	27.3	27.3	27.3
13	143.3	143.6	143.5	28	174.4	174.5	174.5
14	43.0	42.0	42.2	29	29.3	29.2	29.2
15	35.9	35.9	36.0	30	19.1	19.1	19.3

observed. The ^{13}C NMR spectrum of **1** contained eight ^{13}C signals due to the anomeric carbons of sugar moieties at δ 95.7, 99.2, 101.8, 102.2, 105.8, 106.3, 106.8, 111.1, and two methyl ^{13}C signals due to deoxy-sugar moieties at δ 18.7, 18.9. Combined with the results of the HCl-hydrolysis for **1**, it can be deduced that **1** contained three units of glucose, two units of arabinoses and one unit each of xylose, rhamnose and quinovose.

Except for the resonances of proton and ^{13}C belonging to aglycone and sugar moieties, two groups of proton and ^{13}C signals were observed in the ^{1}H and ^{13}C NMR spectra of **1**: proton signals at δ 1.38 (3H, s), 4.70 (2H, s), 5.10 (1H, dd, $J=1.6$, 10.6 Hz), 5.48 (1H, dd, $J=1.6$, 17.2 Hz), 6.04 (1H, dd, $J=10.6$, 17.2 Hz) and 7.16 (1H, t, $J=7.9$ Hz), and δ 1.48 (3H, s), 4.70 (2H, s), 5.18 (1H, d, $J=11.2$ Hz), 5.36 (1H, d, $J=17.4$ Hz), 6.12 (1H, dd, $J=11.2$, 17.4 Hz) and 7.04 (1H, t, $J=7.8$ Hz); for carbon-13 signals see Table II. These resonances were very similar to those of the V′ moiety in *Gleditsia* saponins B and C isolated from the legume of *Gleditsia* japonica cv. 'Saponifera' [6,7], which indicated the presence of two units of (6S)-2-hydroxymethyl-6-methyl-6-hydroxy-2-trans-2,7-octadienoic acid moiety in saponin **1**. The FABMS of **1** in positive ion mode exhibited a quasimolecular ion peak at m/z: 2051 $(M+2+Na)^+$, which was consistent with its molecular weight as calculated for $C_{95}H_{150}O_{46}$ (composed of acacic acid, eight monosaccharides and two monoterpenoids, just the same structure units as those of **2** [1]).

Careful comparison of ^{13}C NMR spectrum for **1** with that for **2** [1] indicated the consistence for most of ^{13}C signals of **1** and **2**, except for those due to quinovose moiety. This suggested common linkage modes for several structure units in **1** and **2** except for those related to quinovose, which was further confirmed by a 2D-^{1}H,^{13}C-long-range experiment

TABLE II ^{13}C NMR data for MT and MT′ moieties of J_1, **1** and **2** (pyridine-d_5)

	MT			MT′		
	J_1	**2**	**1**	J_1	**2**	**1**
1	167.5	167.6	167.5	167.8	167.7	168.0
2	134.9	133.9	133.5	127.9	133.4	133.8
3	145.2	146.3	145.2	143.5	145.3	146.0
4	23.7	24.0	23.7	23.7	24.0	23.7
5	40.9	41.1	40.8	38.6	42.0	41.8
6	79.8	79.8	79.8	79.5	72.3	72.3
7	143.9	144.0	143.7	144.3	146.6	146.5
8	115.2	115.0	115.1	114.3	111.7	111.7
9	56.2	56.6	56.3	12.7	56.6	56.5
10	23.8	23.6	23.9	24.9	28.5	28.5

(HMBC) of **1**. Correlated peaks between G-1 proton (δ 4.87) and C-3 carbon of aglycone (δ 88.8), A′-1 proton (δ 5.14) and G-6 carbon (δ 69.5), and X-1 proton (δ 4.99) and A′-2 carbon (δ 80.3), were observed, which proved the linkage of C-3 sugar chain of **1** to be 3-*O*-xyl-(1 → 2)-ara-(1 → 6)-glc-. The cross peaks between G′-1 proton (δ 6.02) and C-28 carbon (δ 174.5), R-1 proton (δ 5.88) and G′-2 carbon (δ 76.8), A-1 proton (δ 6.23) and R-4 carbon (δ 79.1), and G″-1 proton (δ 5.30) and R-3 carbon (δ 82.1), confirmed the linkage mode of C-28 sugar chain of **1** to be 28-*O*-glc-(1 → 3)-[ara-(1 → 4)-]-rha-(1 → 2)-glc-.

The correlation between H-21 of aglycone (δ 6.29) and MT-1 carbon (δ 167.5) established the attachment of MT moiety directly to C-21 of aglycone. The distinct long range correlation peaks between δ 5.79 (1H, t, $J = 8.1$ Hz) (esterified site) and δ 72.5 (C-qui-2), 74.8 (C-qui-4), and 168.0 (C-MT′-1) were observed in the HMBC spectrum of **1**, suggesting the esterification of MT′ moiety at C-3-OH of quinovose moiety for **1**, instead of C-4-OH for **2**. Meanwhile, the resonances of quinovose moiety observed in the ^{1}H, ^{13}C NMR spectra of **1** were in good agreement with those calculated based on esterification shift values: Observed for proton signals at δ 4.83, 4.05, 5.79, 3.72, 3.68, 1.57; calculated for proton signals at δ 4.82, 3.96, 5.74, 3.69, 3.67, 1.59. Observed for ^{13}C signals at δ 99.2, 72.5, 79.8, 74.8, 72.5, 18.7; calculated for ^{13}C signals at δ 99.2, 72.7, 79.8, 74.3, 72.6, 18.8. This proved the linkage mode between quinovose moiety and MT′ moiety in **1**.

Therefore, saponin **1** was identified as 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl]-21-*O*-{(6S)-2-trans-2-hydroxymethyl-6-methyl-6-*O*-[3-*O*-((6S)-2-trans-2-hydroxymethyl-6-methyl-6-hydroxy-2,7-octadienoyl)-β-D-quinovopyranosyl]-2,7-octadienoyl} acacic acid 28-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl ester(**1**), named as Julibroside J_7, being a new compound, isomeric with Julibroside J_2 in the attached site of MT′. Their structures are shown in Fig. 1. The ^{13}C NMR data of **1** and **2** are given in Tables I–III. The ^{1}H NMR spectral data of **2** are given in the Experimental section for the first time.

EXPERIMENTAL

General Experimental Procedures

Optical rotations were recorded with a Perkin-Elmer 241 using 70% MeOH as solvent. IR spectra were measured on a Perkin-Elmer 983 FT-IR as pressed

TABLE III ^{13}C NMR data of sugar moieties of J_1, **1** and **2** (pyridine-d_5)

	J_1	**1**	**2**		J_1	**1**	**2**
glc				araf			
	106.76	106.8	106.6		111.02	111.1	111.1
	75.60	76.1	77.1		84.42	84.5	84.3
	78.39	78.4	78.5		78.39	78.4	78.5
	72.22	72.1	72.3		85.43	85.4	85.6
	76.07	77.9	77.8		62.55	62.6	62.8
	69.52	69.5	69.6	glc″			
arap					105.73	105.8	105.7
	102.22	102.2	102.4		75.40	75.7	75.7
	80.36	80.4	80.2		78.39	78.4	78.6
	72.53	72.5	72.6		71.79	71.8	72.1
	67.39	67.3	67.5		78.14	78.4	78.5
	64.20	64.2	64.3		62.76	62.8	63.0
xyl				qui			
	106.21	106.3	106.1		99.29	99.2	99.3
	75.40	75.4	75.5		75.59	72.4	75.2
	77.87	77.9	77.3		75.59	79.8	75.6
	70.83	70.9	70.8		77.15	74.8	77.6
	67.16	67.3	67.2		70.17	70.4	70.3
glc′					17.09	18.7	18.8
	95.67	95.7	95.7	qui′			
	76.82	76.8	76.8		99.19		
	78.04	78.2	78.1		75.40		
	71.22	71.2	71.7		78.39		
	79.06	79.1	78.9		76.82		
	61.95	62.0	62.4		72.64		
rha					18.81		
	101.76	101.8	101.7				
	70.53	70.5	70.9				
	82.03	82.0	82.2				
	78.93	79.0	78.9				
	69.15	69.2	69.2				
	18.81	18.9	18.8				

Note: G = 3-*O*-*β*-D-glucopyranosyl = glc, A′ = *α*-L-arabinopyranosyl = arap, X = *β*-D-xylopyranosyl = xyl, G′ = 28-*O*-*β*-D-glucopyranosyl (inner) = glc′, R = *α*-L-rhamnopyranosyl = rha, A = *α*-ara-binofuranosyl = araf, G″ = 28-*O*-*β*-D-glucopyranosyl (outer) = glc″, qui = *β*-D-quinovopyra-nosyl (inner), qui′ = *β*-D-quinovopyranosyl (outer).

KBr disks. 1D- and 2D NMR spectra were recorded using Bruker AM-500 and Varian-300 instruments. FABMS were recorded using a ZABspec mass spectrometer. High performance liquid chromatography was carried out using Gilson automatic system for preparative HPLC with chromatography column: Alltima C_{18} (5 μ, 60A, 22 × 250 mm ID and 10 μ, 60A, 22 × 250 mm ID), using Waters 600 HPLC meter for semi-preparative HPLC with chromatography column: μBondpak C_{18} (6 μ, 60A, 7.8 × 300 mm ID). Macroporous resin D_{101} (Nandai), silica gel (10–40 μ, 200–300 mesh, Qingdao) and Sephadex LH-20 (Pharmacia), Rp C_{18} silica gel (100–200 mesh, Ouya) were used as normal- and reversed-phases, respectively for chromatographic separations. See Introduction Section.

Plant Material

Dried stem bark of *A. julibrissin* was purchased from Mianyang Medicinal Company of Sichuan Province in October 1995. A sample has been deposited in Phytochemistry Department of Beijing Medical University.

Extraction and Isolation

Air-dried powdered stem bark (13.5 kg) was extracted with 95% ethanol. The ethanol residues (1140 g) were suspended in H_2O, then extracted with $CHCl_3$, EtOAc and n-BuOH, respectively. The n-BuOH soluble part was dissolved in MeOH, then poured into acetone dropwise. Precipitates were chromatographed over D_{101} resin column with gradient solvent system (100% $H_2O \rightarrow$ 100% MeOH), MeOH part (248 g) was subjected to silica gel column chromatography eluted with gradient solvent system ($CHCl_3$–CH_3OH–H_2O, 100 : 0 : 0 → 6 : 4 : 1) to afford 68 fractions (500 ml/Fr). Fractions 41–43 were decolorized by active charcoal in MeOH to give a white powder (22.5 g). The white powder (10.5 g) was subjected to repeated Sephadex LH-20 and Rp C_{18} silica gel column chromatography, and finally preparative HPLC (61.5% MeOH/H_2O, 6.0 ml/min, 216 nm) to afford **1** (t_R: 40.5 min) and **2** (t_R: 78.3 min).

Saponin **1** was obtained as a white powder from 61.5% MeOH. $[\alpha]_D^{14}$: −26.8 (c 0.034, 70% MeOH); IR (KBr) ν_{max} (cm^{-1}): 3401 (OH), 2925 (CH), 1708 (C=O), 1640 (C=C), 1565, 1414, 1284, 1073; Positive FABMS m/z: $(M+Na+2)^+$ 2051 (100), $[M+Na+2\text{-}(2glc+rha+ara)]^+$ 1448 (12), $(M+Na+2\text{-}2glc\text{-}rha\text{-}ara\text{-}MT')^+$ 1282 (17). For ^1H NMR data see Results and Discussion. For ^{13}C NMR data see Tables I–III.

Acid hydrolysis of **1** and **2**. A solution of **1** (5.5 mg), and a solution of **2** (14.5 mg), in 2.0 mol/L HCl, were heated to 100°C, refluxed for 12 h, and filtered, respectively. Acacic acid lactone was detected on HPTLC ($CHCl_3$–MeOH, 95 : 5, Rf = 0.20; cyclohexane–acetone, 3 : 1, Rf = 0.24) compared with authentic sample in the precipitates of **1** and **2**. The filtrate was neutralized with Ag_2CO_3 and centrifuged, and then the supernatant evaporated over water bath to dryness. The monosaccharides in the residue of **1** and **2** were detected on PC (n-BuOH–HOAc–H_2O, 4 : 1 : 2, Rf = 0.47 for rha, brown; 0.42 for qui, brown [7]; 0.39 for xyl, red; 0.33 for ara, red; 0.26 for glc, brown; 0.9% aniline–0.05 mol/L oxalic acid, 100°C for 5 min) in comparison with authentic samples.

Julibroside J_2(**2**) was obtained as a white powder from 61.5% MeOH. $[\alpha]_D^{14}$: −24.3 (c 0.054, 70% MeOH); IR (KBr) ν_{max} (cm^{-1}): 3415 (OH), 2919 (CH), 1692 (C=O), 1645 (C=C), 1382, 1070; ^1H NMR (500 MHz,

pyridine-d_5) 1.29, 1.02, 0.99, 1.16, 1.86, 1.06, 1.14 (3H × 7, s, H-23, 24, 25, 26, 27, 29, 30), 5.61 (1H, br s, H-12); 4.85 (1H, d, J = 8.2 Hz, H-glc-1), 5.18 (1H, br s, H-arap-1), 4.99 (1H, d, J = 6.8 Hz, H-xyl-1), 6.01 (1H, d, J = 7.6 Hz, H-glc′-1), 5.88 (1H, s, H-rha-1), 6.18 (1H, s, H-araf-1), 5.25 (1H, d, J = 7.8 Hz, H-glc″-1), 4.79 (1H, d, J = 7.8 Hz, H-qui-1), 1.73 (3H, d, J = 5.7 Hz, H-rha-6), 1.36 (3H, d, J = 6.0 Hz, H-qui-6); 7.03 (1H, t, J = 7.5 Hz, H-MT-3), 6.19 (1H, dd, J = 11.0, 17.8 Hz, H-MT-7), 5.21 (1H, d, J = 11.0 Hz, H-MT-8a), 5.40 (1H, d, J = 17.8 Hz, H-MT-8b), 4.70 (2H, s, H-MT-9), 1.50 (3H, s, H-MT-10); 7.23 (1H, t, J = 7.7 Hz, H-MT′-3), 6.08 (1H, J = 10.7, 17.3 Hz, H-MT′-7), 5.11 (1H, dd, J = 1.6, 10.7 Hz, H-MT′-8a), 5.49 (1H, d, J = 1.6, 17.3 Hz, H-MT′-8b), 4.70 (2H, s, H-MT′-9), 1.36 (3H, s, H-MT′-10). For ^{13}C NMR data see Tables I–III.

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