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A Cytotoxic Saponin with Two Monoterpenoids from *Albizia julibrissin*

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Abstract: A new cytotoxic saponin(**1**), Julibrosides J_{27}, was isolated from the stem barks of *Alibizia. julibrissin* by chromatography, and the structure was elucidated as 3-*O*-β-D-xylopyranosyl-(1→2)-β -D- fucopyranosyl - (1→6) -β - D-glucopyranosyl - 21-*O*-[(6S)-2 -trans-2-hydroxymethyl-6-methyl-6-*O*- [4-*O*-((6S)-2-trans-2-hydroxylmethy 6- methyl - 6-hydroxy)-2,7-octadienoyl-β-D-quinovopy--ranosyl]-2,7-octadienoyl}- acacic acid- 28 -*O*-β-D-glucopranosyl-(1→3)-[(α-L-arabinofuranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyrnosyl ester based on spectral and chemical evidences.

Keyword: *Albizia julibrissin*, Leguminosae, triterpenoid saponin, Julibroside $J_{27,}$ cytotoxic activity.

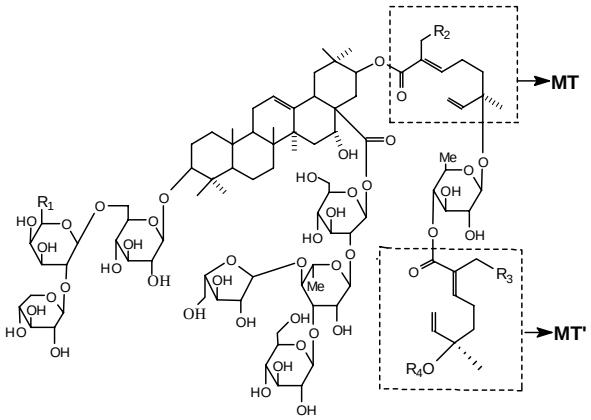
The dried stem barks of *Albizia julibrissin* have been specified in Chinese Pharmacopeia as a sedative agent. In the previous research several novel saponins were reported[1-3].T. Ikeda, *et al*[4] isolated three similar saponins almost at the same time. In our further studies on the 95% ethanol extract from the plant, a new saponin **1** was obtained. This paper involves the isolation and structural elucidation of the new saponin.

Air-dried powdered stem barks (13.5 kg.) were extracted with 95% ethanol. The ethanol extract (1140g) was suspended in H_2O, then extracted with $CHCl_3$, EtOAc and *n*-BuOH,successively. The *n*-BuOH part was subject to Sephadex LH-20 and silica gel column (normal and reversed phases) chromatography, to give a white powder. The white powder was separated by repeated HPLC to afford **1** (164mg).

1 exhibited positive Liebermann-Burchard and Molish reactions, indicating its skeleton of triterpenoid saponin. On acidic hydrolysis with 2.0 mol/L HCl, **1** gave the sapogenin, acacic acid lactone, which was identical with the authentic sample in several TLC conditions. And the structure was confirmed by the ^{1}H NMR data, δ 5.62 (1H, br s, H-12), 3.49 (1H, dd, J=2.2, 12.9 Hz, H-3α), 3.42 (1H, dd, J=1.8, 11.7 Hz, H-18α) and seven single methyl proton signals at δ 1.86, 1.28, 1.15, 1.08, 1.03, 1.01 and 0.96. The ^{13}C NMR signals for genin were in good agreement with those of the aglycone moiety of Julibroside **II** and $\mathbf{J_2}$ (**Table 1**)[4].

On acidic hydrolysis of **1** glucose, xylose, arabinose, quinovose, fucose and rhamnose were detected to be present in the hydrolysate on PC and analytical HPLC in comparison with the authentic samples. The ^{1}H NMR spectrum showed eight anomeric proton signals at 5.08 (1H, d, J=6.8Hz, H-xyl-1), 4.98 (1H, d, J=7.2Hz, H-fuc-1), 4.89 (1H, d, J=6.6Hz, H-glc-1), δ 6.24 (1H, s, H-araf-1), 6.03 (1H, d, J=7.7Hz, H-glc′ -1), 5.87 (1H, br s, H-rha-1), 5.30 (1H, d, J=7.3Hz, H-glc"-1), 4.84 (1H, d, J=5.2Hz, H-qui-1), and three methyl proton signals of deoxysugars at δ 1.74 (3H, d, J=6.2Hz, H-rha-6), 1.50 (3H, d, J=7.2Hz, H-fuc-6) and 1.34 (3H, d, J=6.2Hz, H-qui-6). The ^{13}C NMR spectrum showed the corresponding anomeric carbon-13 signals at δ 106.2,103.3,106.2, 95.7, 101.8, 111.0, 105.7, 99.3 and methyl carbon-13 signals at δ 18.8, 17.1 and 18.8. In comparison of the ^{1}H and ^{13}C NMR spectra for **1** with those of Julibroside **II** (2)[4], except the absence of a group of signals for β -D quinovopyranosy1, all of the signals due to the sugar moieties of **1** were almost superimposable on those of **II** (**Table 2**).

Figure 1 The structure of the Saponins



			R_1	R_2	R_3	R_4
Julibroside	J_{27}	(**1**)	CH_3	OH	OH	H
Julibroside	II	(**2**)	CH_3	H	H	Qui
Julibroside	J_2	(**3**)	OH	OH	OH	H

Two groups of proton signals due to monoterpenoid moieties were observed in ^{1}H NMR spectra of **1**: one group of proton signals at δ 7.03 (1H, t, J=7.8Hz), 6.19 (1H, dd, J=11.3, 17.1Hz), 5.39 (1H, d, J=17.1Hz), 5.20 (1H, d, J=11.3Hz), 4.70 (2H, s), 1.81 (2H, t, J=8.2Hz), 1.48 (3H, s) and another group of proton signals at δ 7.23 (1H, t, J=7.6Hz), 6.07 (1H, dd, J=10.6, 17.1Hz), 5.50 (1H, d, J=17.1Hz), 5.11 (1H, d, J=10.6Hz), 4.75 (2H, s). And two groups of carbon-13 signals for MT and MT′ (see **Table 1**) were observed also, which are identical with those of MT and MT′ of Julibroside **J_2(3)**[3].

The above analysis were confirmed by the FAB-MS results *m/z*: 2065 $[M+Na+2]^+$. Thus, the structure of compound **1** was determined as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl-21-*O*-{(6S)-2-trans-2-hydroxylmethyl-6-methyl–6–*O*- [4 –*O*-((6S) –2– trans –2– hydroxymethyl –6-dimethyl-6-hydroxy-2,7-octadienoyl)-β-D-quinovopyranosyl]-2,7-octadienoyl}-acacic acid –28 -*O*-β-D-gluco-

-pranosyl-(1→3)-[(α-L-arabinofuranosyl-(1→4)]-α-L-rhamnopyranosyl -(1→2)-β-D-glucopyrnosyl ester, named as Julibroside $\mathbf{J_{27}}$.

Table 1. ^{13}C NMR data for sapogenin and MT, MT′ (py-d_5)

C	**1**	**2**	**3**	C	**1**	**2**	**3**
1	39.0	38.9	39.2	MT			
2	26.8	26.8	26.9	1	167.5	168.2	167.6
3	88.8	88.8	88.4	2	133.8	127.8	133.9
4	39.6	39.5	39.7	3	146.5	142.9	146.3
5	56.1	56.0	56.1	4	23.5	23.7	24.0
6	18.7	18.7	18.4	5	41.0	40.2	41.1
7	33.6	33.6	33.8	6	79.7	79.7	79.8
8	40.2	40.1	40.3	7	144.0	143.8	144.0
9	47.2	47.1	47.3	8	114.9	115.5	115.1
10	37.1	37.0	37.3	9	56.3	12.6	56.6
11	23.7	23.9	23.9	10	24.0	23.5	23.6
12	123.1	123.1	123.1				
13	143.3	143.7	143.5				
14	42.0	41.9	42.2				
15	35.9	35.6	36.0				
16	73.9	73.6	73.9	MT′			
17	51.6	51.7	51.8	1	167.6	168.2	167.7
18	40.8	40.8	40.9	2	133.4	128.3	133.4
19	47.9	47.7	48.1	3	145.2	143.6	145.3
20	35.4	35.2	35.5	4	23.9	23.7	24.0
21	77.2	76.5	76.9	5	41.9	40.4	42.0
22	36.4	36.2	36.5	6	72.2	80.0	72.3
23	28.2	28.2	28.4	7	146.5	143.8	146.6
24	15.9	15.8	15.9	8	111.7	115.2	111.7
25	17.1	17.0	17.5	9	56.3	12.6	56.6
26	17.4	17.3	17.5	10	28.5	23.6	28.5
27	27.3	27.2	27.3				
28	174.4	174.8	174.5				
29	29.2	29.0	29.2				
30	19.1	19.1	19.3				

Julibroside $\mathbf{J_{27}}$ exhibited good inhibitory action against KB cell line with ED_{50} 0.6μM, and good inhibitory action against Bel cell line with ED_{50} 5.0μM. But it showed no marked inhibitory action against HL-60 cell line with the ED_{50} more than 20μM *in vitro*.

Table 2 ^{13}C NMR data for sugar moieties (py-d_5)

C	**1**	**2**	**3**	C	**1**	**2**	**3**
glc 1	106.2	106.4	106.6	rha 1	101.8	101.7	101.7
2	76.8	75.0	77.1	2	70.6	70.4	70.9
3	78.4	77.8	78.5	3	82.0	81.7	82.2
4	72.6	71.9	72.3	4	79.0	78.8	78.9
5	77.2	77.3	77.8	5	69.2	69.1	69.2
6	69.5	69.6	69.6	6	18.8	18.8	18.8
fuc 1	103.3	103.1	arap	araf 1	111.0	110.5	111.1
2	82.0	81.8	102.4	2	84.4	83.8	84.3
3	75.4	74.8	80.2	3	78.4	78.0	78.5
4	72.2	72.3	72.6	4	85.4	85.1	85.6
5	71.3	71.2	67.5	5	62.6	62.3	62.8
6	17.1	17.1	64.3	glc″ 1	105.7	105.4	105.7
xyl 1	106.2	106.4	106.1	2	75.3	75.1	75.7
2	75.6	75.6	75.5	3	78.4	77.8	78.6
3	78.2	77.8	77.3	4	71.7	71.2	72.1
4	70.8	70.4	70.8	5	78.4	77.8	78.5
5	67.3	66.9	67.2	6	62.8	61.8	63.0
glc′ 1	95.7	95.3	95.7	qui 1	99.3	99.1	99.3
2	77.1	76.3	76.8	2	75.5	75.5	75.2
3	77.5	77.6	78.1	3	75.4	75.4	75.6
4	71.1	70.8	71.7	4	77.1	77.1	77.6
5	79.0	78.4	78.9	5	70.6	70.1	70.3
6	62.0	62.3	62.4	6	18.8	18.3	18.8
				qui′ 1		99.0	
				2		75.1	
				3		78.0	
				4		77.0	
				5		72.3	
				6		18.3	

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