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Bioorganic & Medicinal Chemistry Letters 15 (2005) 4493–4495
Bioorganic & Medicinal Chemistry Letters

An antitumor compound julibroside J_{28} from *Albizia julibrissin*

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Received 15 April 2005; revised 28 June 2005; accepted 7 July 2005
Available online 19 August 2005

Abstract—A new triterpenoid saponin, julibroside J_{28} (**1**), was isolated from the stem bark of *Albizia julibrissin* Durazz (Leguminosae) by using chromatographic method. The structure of **1** was established by spectroscopic methods. **1** displayed significant antitumor activity in vitro against PC-3M-1E8, Bel-7402, and HeLa cancer cell lines at 10 μM assayed by SRB method.

Albizia julibrissin (Leguminosae) has been recorded in Chinese Pharmacopoeia as a sedative and an anti-inflammatory drug for treating swelling and pain of the lungs, skin ulcers, and wounds.[1] In the previous research, the novel and complex triterpenoid saponin with cytotoxic activity was isolated and identified.[2] On our continuing study, a new saponin obtained from the *n*-BuOH soluble part of the 95% ethanol extracts from the stem barks of *A. julibrissin* showed significant inhibitory activity in vitro against human tumor cell lines. Isolation[5] of the active extract led to the separation of compound **1** as the major novel active principle. The structure of **1**, named julibroside J_{28}, was determined by NMR spectra, including ^{1}H–^{1}H COSY, HSQC, TOCSY, and HMBC techniques.[6] Compound **1** had significant antitumor activity against PC-3M-1E8, Bel-7402, and HeLa cancer cell lines in vitro. We herein report the isolation and structural characterization of julibroside J_{28}.

Julibroside J_{28} (**1**), white powder, gave positive Liebermann–Burchard reaction and Molish reaction. MALDI-TOF-MS showed the quasi-molecular ion peak at *m*/*z* 2219 $[M+1+Na]^{+}$. Its ^{1}H NMR spectrum showed seven angular methyl signals at δ 0.94 (3H, s), 0.99 (3H, s), 1.03 (3H, s), 1.05 (3H, s), 1.18 (6H, s), and 1.89 (3H, s), one olefinic proton at δ 5.60 (1H, br s), and sugar proton signals at δ 3.5–6.4. ^{13}C NMR spectrum indicated two olefinic carbon signals at δ 143.3 and 123.1, suggesting that **1** was an oleanane type triterpenoid saponin. In a comparison of the ^{13}C NMR signals for aglycone of **1** with those of known saponin julibroside III (**2**)[3] (Table 1), all signals due to the aglycone of **1** were almost superimposable with those of **2**, indicating the aglycone of **1** was same as that of **2**, which was acacic acid (3β,16α,21β-trihydroxyolean-12-ene-28-oic acid) and its 3,21-hydroxy groups and 28-carbonyl group carried a sugar moiety, respectively. On acidic hydrolysis, **1** furnished glucose, glucosamine hydrochloride, fucose, xylose, rhamnose, arabinose, and quinovose, which were identified by co-TLC with authentic samples. On acidic hydrolysis, amino sugar gave glycosamine hydrochloride. ^{13}C NMR spectrum gave nine anomeric carbon signals at δ 95.7, 99.3, 99.4, 101.8, 103.4, 104.8, 105.8, 107.0, and 111.1. The anomeric proton signals at δ 4.83 (1H, d, J = 8.0 Hz), 4.86 (1H, d, J = 8.0 Hz), 5.03 (1H, d, J = 8.5 Hz), 4.99 (1H, d, J = 8.5 Hz), 5.08 (1H, d, J = 6.0 Hz), 5.35 (1H, d, J = 7.0 Hz), 5.91 (1H, br s), 6.06 (1H, d, J = 7.5 Hz), and 6.28 (1H, br s) were assigned by direct correlation from HSQC. On the basis of the ^{1}H and ^{13}C NMR data of **1**, the anomeric configurations of the sugar moieties were determined as β-configuration for glucose, 2-deoxy-2-acetamidoglucose, fucose, xylose, and quinovose, and α-configuration for rhamnose and arabinose. In the ^{13}C NMR spectrum of **1**, all of the signals due to sugar moieties of **1** were identical with those of julibroside III (**2**)[3] (Table 1), indicating that the sugar moieties of **1** were the same as those of julibroside III. Except the signals of the aglycone and sugar moieties, there were

Keywords: *Albizia julibrissin*; Julibroside J_{28}; Antitumor activity.
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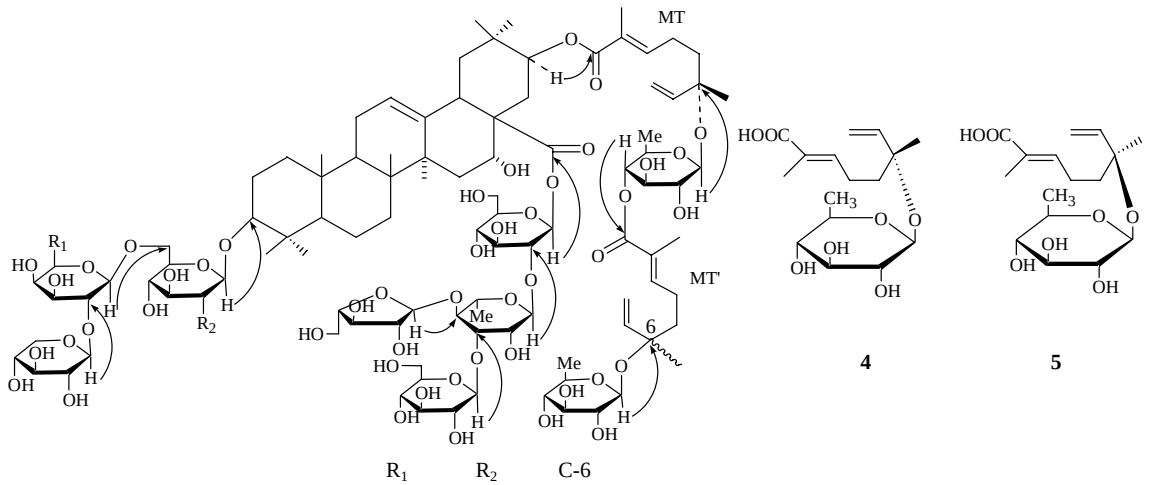
doi:10.1016/j.bmcl.2005.07.015

Table 1. ^{13}C NMR data of compounds **1**–**5** (Py-d_5)

C	2	1	C	2	1	C	2	1	C	4	5	2	3	1
1	38.7	38.8	C-3			C-28			MT					
2	26.5	26.6	glc 1	104.6	104.8	glc′ 1	95.5	95.7	1			167.8	167.8	167.8
3	88.8	88.8	2	57.8	57.9	2	76.7	76.9	2			128.6	127.9	127.9
4	39.3	39.4	3	75.8	75.9	3	78.0	78.2	3			142.3	142.3	142.3
5	55.9	56.0	4	72.1	72.2	4	71.1	71.2	4			23.7	23.8	23.6
6	18.6	18.4	5	77.4	77.5	5	78.9	79.1	5			40.5	40.5	40.5
7	33.6	33.6	6	69.8	70.0	6	61.9	61.9	6			79.8	79.4	79.7
8	40.1	40.1	C═O	170.1	170.0				7			144.1	144.0	144.0
9	47.1	47.1	CH_3	23.7	23.6	rha 1	101.7	101.8	8			115.0	115.0	115.1
10	37.0	37.1				2	70.5	70.6	9			12.7	12.7	12.7
11	23.8	23.8	fuc 1	103.3	103.4	3	81.9	82.0	10			23.8	23.5	23.9
12	123.0	123.1	2	82.1	82.2	4	79.0	79.1	MT′					
13	143.2	143.3	3	75.1	75.3	5	69.0	69.1	1	170.6	170.7	167.8	167.8	167.7
14	41.9	42.0	4	72.4	72.5	6	18.7	18.9	2	129.0	129.1	128.2	128.6	128.5
15	35.8	36.0	5	71.7	71.8				3	142.4	142.2	144.0	143.5	143.6
16	73.8	73.9	6	17.1	17.2	araf 1	110.9	111.1	4	23.6	23.8	23.7	23.7	23.6
17	51.6	51.6				2	84.3	84.5	5	38.8	40.6	40.5	38.7	38.4
18	40.9	41.0	xyl 1	106.8	107.0	3	78.3	78.4	6	79.5	79.6	79.5	79.8	79.4
19	47.7	47.8	2	75.8	75.8	4	85.4	85.5	7	144.4	144.2	144.1	144.4	144.4
20	35.2	35.3	3	78.3	78.4	5	62.6	62.7	8	114.1	114.8	114.8	114.1	114.2
21	76.8	77.2	4	70.7	70.8				9	12.9	12.8	12.7	12.8	12.8
22	36.3	36.4	5	67.0	67.1				10	24.7	23.9	23.8	24.8	24.9
23	28.1	28.1												
24	17.0	17.1	C-21											
25	15.7	15.8	qui 1	99.2	99.4	qui′ 1	99.1	99.3	glc″ 1			105.7		105.8
26	17.2	17.3	2	75.5	75.6	2	75.5	75.5	2			75.3		75.5
27	27.2	27.3	3	75.5	75.6	3	78.3	78.4	3			78.3		78.4
28	174.4	174.4	4	77.1	77.0	4	77.0	77.0	4			71.2		71.3
29	29.1	29.2	5	70.1	70.2	5	72.5	72.7	5			78.1		78.1
30	19.0	19.1	6	18.3	18.4	6	18.6	18.7	6			62.4		62.5

another 20 carbon signals, indicating **1** had two monoterpene moieties (MT and MT′). ^1H NMR spectrum of MT and MT′ showed 4 methyl proton signals at δ 1.93 (3H, s), 1.83 (3H, s), 1.53 (3H, s), 1.45 (3H, s), two olefinic proton signals at δ 7.10 (1H, t, $J = 7.5$ Hz) and 6.88 (1H, t, $J = 7.5$ Hz), and two groups of one-substituted olefin proton signals, one group at δ 6.22 (1H, dd, $J = 17.0$, 11.0 Hz), 5.25 (1H, d, $J = 11.0$ Hz), and 5.45 (1H, d, $J = 17.0$ Hz), and the other group at δ 6.31 (1H, dd, $J = 18.0$, 11.0 Hz), 5.18 (1H, d, $J = 11.0$ Hz), and 5.41 (1H, d, $J = 18.0$ Hz). ^1H and ^{13}C NMR data of the two monoterpenes of **1** indicated that the two monoterpenes had different configurations with that of **2**. One- and two-dimensional NMR techniques including ^1H NMR, ^{13}C NMR, DEPT, ^1H–^1H COSY, HSQC, TOCSY, and HMBC spectra, permitted assignments of all ^1H and ^{13}C NMR data of the two monoterpenes. Saponins **1** and **2** possessed markedly



	R_1	R_2	C-6
Julibroside J_{28} (**1**)	CH_3	NHAc	R
Julibroside III (**2**)	CH_3	NHAc	S
Julibroside J_{14} (**3**)	H	OH	R

Figure 1. The structures of **1**–**5** and the HMBC of **1**.

different ^{13}C NMR data due to differences in MT′ group (Table 1). A comparison of the ^{13}C NMR data of **1** with those of **2** showed that the signal of C-5 and C-10 of **1** underwent an upfield shift of 2.1 ppm and a downfield shift of 1.1 ppm, respectively. The above differences between two saponins were quite similar to the ^{13}C NMR data of (6*R*)-menthiafolic acid-6-*O*-β-D-quinovoside (**4**) and (6*S*)-menthiafolic acid-6-*O*-β-D-quinovoside (**5**)[4] (see Fig. 1), which revealed the configurations of the two monoterpene moieties were C-6 (*S*) and C-6 (*R*), respectively. The ^{1}H and ^{13}C NMR data of monoterpene moieties of **1** were in agreement with those of julibroside J_{14} (**3**)[7] (Table 1). The linkage modes for the above structural units (aglycone, nine sugars, MT, and MT′) were established by HMBC experiments of **1** (Fig. 1). Finally, the structure of **1** was determined as 3-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-fucopyranosyl-(1 → 6)-β-D-2-deoxy-2-acetamidoglucopyranosyl]-21-*O*-{(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-[4-*O*-((6*R*)-2-*trans*-2,6-dimethyl-6-*O*-β-D-quinovopyranosyl)-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. Compound **1** was a new saponin, named julibroside J_{28}.

Julibroside J_{28} (**1**) showed significant activity against PC-3M-1E8, Bel-7402, and HeLa cancer cell lines; the inhibitory rates were 80.47, 70.26, and 58.53%, respectively, at 10.0 μM assayed by SRB method.

Acknowledgments

This project was supported by National Natural Science Foundation of China (Grant Nos. 29632050 and 20432030).

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5. *Extraction and isolation*. The air-dried powder of the stem barks of *Albizia julibrissin* Durazz. (8.0 kg) was extracted with hot water (90–100 °C). The water extract was concentrated in vacuo to yield a residue that was partitioned with *n*-BuOH and water. The *n*-BuOH extract (120.0 g) was chromatographed over HP-20 macroporous resin column by elution with gradient solvent system (100% H_2O → 100% MeOH), MeOH part (30.0 g) was subjected to silica gel column chromatography, eluted with $CHCl_3$–CH_3OH–H_2O (65:35:10) to afford four fractions (Fr 1–Fr 4). Fr 3 was subjected to Rp C_{18} silica gel column chromatography and preparative HPLC (74:26 MeOH–H_2O, 2.2 mL/min, 216 nm detection) to afford **1** (9 mg).
6. Julibroside J_{28}, white powder, MALDI-TOF-MS *m/z* 2219 $[M+1+Na]^+$; ^{1}H NMR (500 MHz, py-d_5): δ 1.18, 0.99, 0.94, 1.18, 1.89, 1.03, 1.05 (each 3H, s, H-23, 24, 25, 26, 27, 29, 30), 5.60 (1H, br s, H-12), 5.03 (1H, d, J = 8.5 Hz, glc H-1), 4.99 (1H, d, J = 8.5 Hz, fuc H-1), 5.08 (1H, d, J = 6.0 Hz, xyl H-1), 6.06 (1H, d, J = 7.5 Hz, glc′ H-1), 5.91 (1H, br s, rha H-1), 6.28 (1H, br s, araf H-1), 5.35 (1H, d, J = 7.0 Hz, glc″ H-1), 4.86 (1H, d, J = 8.0 Hz, qui H-1), 4.83 (1H, d, J = 8.0 Hz, qui′ H-1), 1.48 (3H, d, J = 5.5 Hz, fuc H-6), 1.35 (3H, d, J = 6.0 Hz, qui H-6), 1.78 (3H, d, J = 5.5 Hz, rha H-6), 1.59 (3H, d, J = 5.0 Hz, qui′ H-6) 6.88 (1H, t, J = 7.5 Hz, MT H-3), 6.22 (1H, dd, J = 11.0, 17.0 Hz, MT H-7), 5.25 (1H, d, J = 11.0 Hz, MT H-8a), 5.45 (1H, d, J = 17.0 Hz, MT H-8b), 1.83 (3H, s, MT H-9), 1.53 (3H, s, MT H-10), 7.10 (1H, t, J = 7.5 Hz, MT′ H-3), 6.31 (1H, dd, J = 11.0, 18.0 Hz, MT′ H-7), 5.18 (1H, d, J = 11.0 Hz, MT′ H-8a), 5.41 (1H, d, J = 18.0 Hz, MT′ H-8b), 1.93 (3H, s, MT′ H-9), 1.45 (3H, s, MT′ H-10). ^{13}C NMR (125 MHz, py-d_5) data, see Table 1.
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