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Diastereoisomeric saponins from *Albizia julibrissin*

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Abstract—The structures of four new diastereoisomeric triterpenoidal saponins Julibroside J_5, J_8, J_{12} and J_{13} (**1**–**4**) isolated from *Albizia julibrissin* Durazz. (Leguminosae) have been determined on the basis of comprehensive spectroscopic analysis and chemical degradation. Julibroside, J_8 and J_{13} showed marked cytotoxic activities against Bel-7402 cancer cell line at 100 μg/mL.

Keywords: *Albizia julibrissin*; Julibroside J_5; Julibroside J_8; Julibroside J_{12}; Julibroside J_{13}; Diastereoisomer; Cytotoxicity

1. Introduction

Albizia julibrissin (Leguminosae) has been recorded in Chinese Pharmacopoeia as a sedative drug and an anti-inflammatory agent for treating swelling and pain of the lungs, skin ulcers and wounds. Previously, Ma et al.[1] and Ikeda et al.[2] reported four new triterpenoid saponins with the (6*S*)-configuration in the monoterpene moieties. Now we report the isolation and structural elucidation of other four new triterpenoid saponins named as Julibroside J_5, J_8, J_{12}, and J_{13} (**1**–**4**), **1** and **3** with both (6*S*) and (6*R*)-configurations in the monoterpene moieties. Julibroside J_8 and J_{13} showed marked cytotoxic activities against Bel-7402 cancer cell line at 100 μg/mL.

2. Results and discussion

The 95% ethanol extract from stem barks of *A. julibrissin* was suspended in water and extracted successively with $CHCl_3$, EtOAc, *n*-BuOH. The *n*-BuOH soluble part was chromatographed on D_{101} macroporous resin, Sephadex LH-20 and silica gel columns to afford colorless powders (Frs 41–43). Two pairs of isomers, **1** and **2**, **3** and **4** were obtained from Frs 41–43 by repeated reverse phase C18 column chromatography and preparative HPLC.

Compound **1**, a white powder, gave a positive Liebermann–Burchard reaction. The ^{1}H NMR spectrum showed seven angular methyl signals at δ 1.30, 1.02, 0.96, 1.15, 1.89, 1.03, 1.07 (each 3H, s) and sugar proton signals at 3.4–6.3. The data suggested **1** was a triterpenoid saponin. On acidic hydrolysis, **1** furnished the aglycone which was identical with an authentic sample, acacic acid lactone on high-performance thin layer chromatography, and on PC the resulting sugars were identified as glucose, fucose, xylose, rhamnose, arabinose and quinovose. ^{13}C NMR spectrum gave nine anomeric carbon signals at 95.7, 99.2, 99.3, 101.8, 103.4, 105.8, 106.7, 106.9 and 111.0. The anomeric proton signals δ 6.04 (d, J 7.5 Hz), 4.84 (d, J 5.9 Hz), 4.82 (d, J 7.8 Hz), 5.88 (br s), 4.99 (d, J 7.8 Hz), 5.32 (d, J 7.9 Hz), 4.94 (d, J 8.5 Hz), 5.08 (d, J 7.0 Hz) and 6.26 (br s) were assigned by direct correlation from C–H COSY. Based on the ^{1}H and ^{13}C NMR data of **1**, the configurations of anomeric carbons in the sugar moieties were determined as β-configuration for glucose, fucose,

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xylose, and quinovose moieties, and α-configuration for rhamnose and arabinose moieties. Except for the resonances of proton and carbon-13 belonging to aglycone and sugar moieties, two groups of proton and carbon-13 signals of the monoterpenoids (MT, MT′) were observed in ^{1}H and ^{13}C NMR spectra of **1** (see Tables 1–2). In the ^{13}C NMR spectrum of **1**, the signals of the aglycone, monoterpene and sugars moieties were almost superimposable on those of Julibroside J_1 (J_1),[1,7] except that **1** gave the signals due to fucose, which instead of the signals due to arabinose of J_1, indicating that the aglycone, monoterpene moieties of **1** were the same as those of J_1. In a comparison of the ^{13}C NMR data of **1** with those of julibrosid II (**II**),[2] all of the signals due to sugar moieties of **1** were identical with those of **II**, indicating that the sugars of **1** were the same as those of Julibroside II (see Fig. 2). This conclusion was supported by 2D NMR and MALDI-TOF-MS experimental results. The MALDI-TOF-MS showed the quasi-molecular ion peak at *m/z* 2193 $[M+Na]^+$. 1D and 2D NMR techniques (^{1}H, ^{13}C NMR, DEPT, H–H COSY, HSQC, TOCSY, HMBC) permitted assignments of ^{1}H and ^{13}C NMR data (Tables 1–3). The linkage modes for the above structural units (aglycone, nine sugars, MT and MT′) were established by HMBC (see Table 4, Fig. 2) and NOESY experiments of **1**.

On alkaline hydrolysis with $NaHCO_3/H_2O$ **1** gave **1B** (Fig. 1). The ^{1}H, ^{13}C NMR signals of **1B** were identical with those of (6*R*)-menthiafolic acid-6-*O*-β-D-quinovoside (compound **2a**).[3] Compound **1B** was hydrolyzed with β-glucosidase (from almonds) to afford **1C** (Fig. 1). The absolute configuration for C-6 of outer monoterpene (**MT′**, see Fig. 2) was determined to be 6*R* by the rotation of **1C**, $[\alpha]_D^{14}$ −14.6 in a comparison with that of (6*R*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid (compound **12**).[4] ^{1}H NMR data of **1C** was identical with those of (6*R*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid. Then the structure of **1** was determined as 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl]-21-*O*-{(6*S*)-2-*trans*-2-hydroxymethyl-6-methyl-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 and named Julibroside J_5.

Table 2. ^{13}C NMR data of monoterpenoidal moieties (Py-d_5)

	1	2	3	4
MT				
1	167.5	167.6	167.6	167.6
2	133.8	133.9	133.9	133.9
3	145.2	145.2	145.1	145.2
4	23.6	23.9	23.8	23.9
5	40.9	40.5	40.4	40.5
6	79.7	79.8	79.8	79.8
7	143.9	144.4	144.1	144.1
8	115.1	115.1	115.0	115.0
9	56.3	56.4	56.4	56.4
10	23.9	23.7	23.6	23.7
MT′				
1	167.8	167.8	167.8	167.8
2	127.9	128.0	128.0	128.0
3	143.4	143.9	143.9	143.9
4	23.9	23.9	23.8	23.8
5	38.5	40.9	38.7	40.9
6	79.7	79.5	79.4	79.5
7	144.4	144.4	144.3	144.3
8	114.2	114.8	114.2	114.8
9	12.8	12.7	12.8	12.7
10	24.8	23.9	24.8	23.7

Compound **2** was obtained with a longer retention time than that of **1** by HPLC: t_R of **1** = 86.2 min. t_R of **2** = 97.5 min (67–68.5% MeOH). The MALDI-TOF-MS, ^{1}H and ^{13}C NMR spectra of **2** were good agreement with those of **1**, except that proton and carbon-13 signals due to MT′ group (Table 2). A comparison of the ^{13}C NMR data of **2** with those of **1** showed that the sig-

Table 1. ^{13}C NMR data of aglycone of **1**–**4** and ^{1}H NMR of monoterpene moieties of **1** and **2** (Py-d_5)

Carbon	1	2	3	4	Carbon	1	2	3	4		1	2
1	38.9	39.0	39.0	39.1	16	73.9	73.9	73.9	73.9	*MT*		
2	26.8	26.8	26.9	26.6	17	51.6	51.7	51.7	51.7	3	7.02 (t, 7.4)	7.01 (br s)
3	88.5	88.4	88.9	88.9	18	40.8	40.9	40.9	40.9	7	6.19 (dd, 10.9, 17.6)	6.20 (dd, 11.2, 17.6)
4	39.6	39.7	39.7	39.4	19	47.8	47.9	47.9	48.0	8a	5.20 (d, 10.9)	5.21 (d, 11.2)
5	56.0	56.1	56.1	56.1	20	35.4	35.5	35.5	35.5	8b	5.39 (d, 17.6)	5.40 (d, 17.6)
6	18.8	18.4	18.4	18.4	21	76.8	76.9	76.9	76.9	9	4.71 (s)	4.70 (s)
7	33.6	33.7	33.7	33.7	22	36.4	36.5	36.5	36.5	10	1.50 (s)	1.50 (s)
8	40.2	40.2	40.2	40.2	23	28.2	28.3	28.2	28.2	*MT′*		
9	47.2	47.2	47.2	47.2	24	17.2	17.2	17.0	17.1	3	7.09 (t, 7.0)	7.01 (br s)
10	37.1	37.2	37.2	37.2	25	15.9	15.9	15.9	15.8	7	6.32 (dd, 11.1, 17.1)	6.20 (dd, 11.2, 17.6)
11	23.7	23.7	23.8	23.8	26	17.4	17.4	17.4	17.4	8a	5.18 (d, 11.1)	5.21 (d, 11.2)
12	123.1	123.1	123.1	123.1	27	27.3	27.3	27.3	27.3	8b	5.33 (d, 17.7)	5.41 (d, 17.6)
13	143.6	143.4	143.4	143.4	28	174.4	174.4	174.5	174.5	9	1.93 (s)	1.88 (s)
14	42.0	42.1	42.1	42.1	29	29.2	29.2	29.2	29.2	10	1.45 (s)	1.53 (s)
15	35.9	36.0	36.0	36.0	30	19.2	19.2	19.2	19.2			

Note: MT and MT′ are shown in Figure 2.

Table 3. NMR data of sugars moieties of **1**–**4** (Py-d_5)

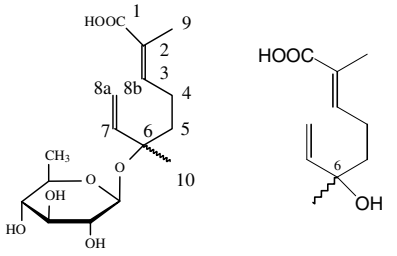
	1			2	3	4
	^{13}C	^{1}H		^{13}C		
C-3 Glc*p*						
1	106.7	4.94	(d, 8.5)	106.7	104.7	104.6
2	76.8	4.05		76.9	58.1	58.6
3	78.4	4.19		78.4	75.6	75.6
4	72.2	4.18		72.6	72.6	72.6
5	77.2	4.11		77.8	77.5	77.5
6	70.0	4.74	4.38	69.5	69.6	69.6
2-NHAc-						
C=O					170.1	170.1
CH_3					23.8	23.8
Fuc*p*						
1	103.4	4.99	(d, 7.8)	103.4	103.4	103.4
2	82.1	4.43		82.1	82.1	82.1
3	75.4	4.01		75.4	75.6	75.6
4	71.8	4.18		71.8	72.3	72.3
5	71.3	3.75		72.3	72.0	71.9
6	17.2	1.47		17.2	17.2	17.2
Xyl*p*						
1	106.9	5.08	(d, 7.0)	106.8	106.8	106.8
2	75.8	4.03		75.6	75.5	75.6
3	78.2	4.08		77.2	77.2	77.2
4	70.8	4.12		70.8	70.8	70.9
5	67.2	4.48	4.06	67.2	67.1	67.3
C-28 Glc*p*′						
1	95.7	6.04	(d, 7.5)	95.7	95.7	95.7
2	76.8	3.99		76.9	76.9	76.9
3	77.1	4.15		77.5	78.1	77.5
4	71.2	4.18		71.2	71.4	71.4
5	79.4	3.93		79.0	79.0	79.0
6	62.0	4.32	4.20	62.1	62.1	62.2
Rha*p*						
1	101.8	5.88	(br s)	101.8	101.8	101.8
2	70.5	5.18		70.6	70.6	70.8
3	82.0	4.93		82.0	82.0	81.9
4	79.0	4.47		78.9	79.0	79.0
5	69.2	4.53		69.2	69.2	69.2
6	18.8	1.75		18.7	18.8	18.8
Ara*f*						
1	111.0	6.26	(br s)	111.1	111.1	111.1
2	84.5	4.98		84.4	84.4	84.4
3	78.2	4.80		78.4	78.5	78.5
4	85.4	4.73		85.5	85.5	85.5
5	62.8	4.24	4.16	62.7	62.7	62.7
Glc*p*″						
1	105.8	5.32	(d, 7.9)	105.7	105.8	105.7
2	75.2	3.98		75.6	75.7	75.6
3	78.4	4.21		78.4	78.2	78.2
4	71.7	4.08		71.9	71.8	71.9
5	78.3	3.94		78.4	78.5	78.5
6	62.6	4.50	4.19	62.9	62.0	62.9
C-21						
Qui*p*						
1	99.3	4.82	(d, 7.8)	99.3	99.3	99.3
2	75.6	4.01		75.6	75.3	75.4
3	75.6	4.21		75.6	75.7	75.7
4	77.9	5.35		77.2	77.2	77.2
5	70.2	3.68		70.2	70.2	70.2
6	18.4	1.34		18.8	18.8	18.8

Table 3 (*continued*)

	1			2	3	4
	^{13}C	^{1}H		^{13}C		
Qui*p*′						
1	99.2	4.84	(d, 5.9)	99.3	99.2	99.3
2	75.4	3.98		75.2	75.2	75.2
3	78.4	4.12		78.2	78.5	78.2
4	77.5	3.71		76.9	76.8	76.9
5	72.0	3.67		72.6	72.7	72.6
6	18.7	1.53		18.7	18.8	18.8

Table 4. The key correlations in HMBC experiment of **1**

^{1}H	^{13}C
4.94 (Glc*p* 1-H)	88.5 (genin 3-C)
4.99 (Fuc*p* 1-H)	70.0 (Glc*p* 6-C)
5.08 (Xyl*p* 1-H)	75.4 (Fuc*p* 2-C)
6.04 (Glc*p*′ 1-H)	174.4 (genin 28-C)
5.88 (Rha*p* 1-H)	96.8 (Glc*p*′ 2-C)
6.26 (Ara*f* 1-H)	79.0 (Rha*p* 4-C)
5.32 (Glc*p*″ 1-H)	82.0 (Rha*p* 3-C)
6.30 (genin 21-H)	167.5 (MT 1-C)
4.82 (Qui*p* 1-H)	79.7 (MT 6-C)
5.35 (Qui*p* 4-H)	167.8 (MT′ 1-C)
4.84 (Qui*p*′ 1-H)	79.7 (MT′ 6-C)

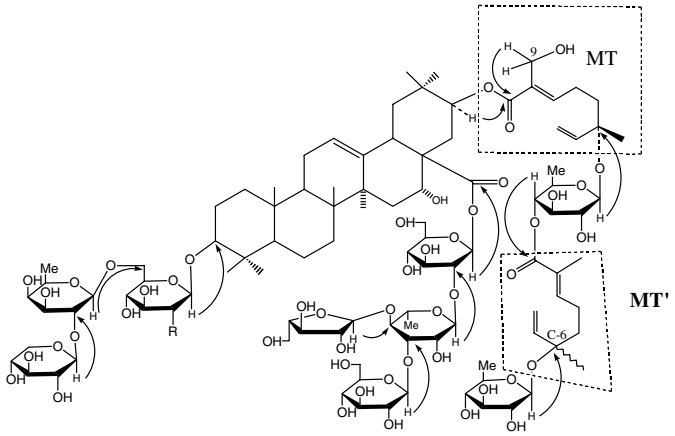


1B. 6*R* ; **2B.** 6*S** **1C.** 6*R* ; **2C.** 6*S*

Figure 1. The hydrolysis products of **1** and **2**.

nals for C-5 and C-10 of MT′ of **2** undergo a downfield shift of 2.4 ppm and upfield shift of 0.9 ppm (see Table 2).

On alkaline hydrolysis as above **2** gave **2B**. The ^{1}H, ^{13}C NMR signals of **2B** were identical with those of (6*S*)-menthiafolic acid-6-*O*-β-D-quinovoside (compound **2b**).[6] Compound **2B** was hydrolyzed with β-glucosidase to afford **2C**. The absolute configuration for C-6 of MT′ was determined to be 6*S* by a comparison of its rotation, $[\alpha]_D^{14}$ +18.2 with that of (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoyl acid ((6*S*)-menthiafolic acid) (IV or 10).[5,6] The structure of **2** was determined as 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl]-21-*O*-{(6*S*)-2-trans-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-((6*S*)-2-*trans*-2,6-dimethyl-6-*O*-(β-D-quinovopyanosyl)-2,7-octadienoyl)-β-D-quinovopyranosyl]-2,7-octadienoyl}-acacic acid-28-



1. R=OH, 6*R* **3.** R=NHAc, 6*R*
2. R=OH, 6*S* **4.** R=NHAc, 6*S*

Figure 2. The structures of **1**–**4** and main HMBC of **1**.

O-β-D-glucopyranosyl-(1→3)-[α-L-arabinofuranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester and named Julibroside J_8. Compounds **1** and **2** were a pair of diastereoisomers, and possessed markedly different ^{13}C NMR signals due to C-5 and C-10 of MT′, which were the same as those of compounds **2a** and **2b** (see experimental, in py-d_5).[3] The (6*S*)-configuration in outer monoterpene moiety of Julibroside J_1 was revised as (6*R*).[7]

Compound **3** was obtained as a white powder. In a comparison of the ^1H and ^{13}C NMR spectra of **3** with those of **1**, the data were identical (Tables 1–3), except for carbon-13 signals 104.7 (C-1), 58.1 (C-2), δ 170.1 (C═O), 23.8 (**CH**$_3$COHN–) due to 3-*O*-β-D-2-deoxy-2-acetamidoglucopyranosyl moiety of **3** instead of the signals δ 106.7 (C-1), 76.8 (C-2) due to 3-*O*-β-D-glucopyranosyl moiety of **1**, suggesting that the inner sugar linked to genin via the C-3 hydroxy group was 2-deoxy-2-acetamidoglucopyranose. In a comparison of the ^{13}C NMR data of **3** with those of Julibroside III (**III**),[2] all of the signals due to sugar moieties of **3** were identical with those of **III**, indicating that the sugars of **3** were the same as those of Julibroside III. This conclusion was supported by MS data. The MALDI-TOF -MS gave the quasi-molecular ion peak at *m*/*z* 2235 $[M+H+Na]^+$. Thus, the structure of **3** 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-hydroxymethyl-6-methyl-6-*O*-[4-*O*-((6*R*)-2-*trans*-2,6-dimethyl-6-β-D-(quinovopyanosyl)-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 and named Julibroside J_{12} (see Fig. 2).

Compound **4** was obtained with a longer retention time than that of **3** by HPLC: t_R of **3** = 72.1 min. t_R of **4** = 79.0 min (67–68.5% MeOH). The MALDI-TOF-MS, ^1H and ^{13}C NMR spectra of **4** were in good agreement with those of **3**, except that proton and carbon-13 signals due to MT′ group (Table 2). A comparison of the ^{13}C NMR data of **4** with those of **3** showed that the signals for C-5 and C-10 of MT′ of **4** undergo a downfield shift of 2.2 ppm and upfield shift of 1.1 ppm. The structure of **4** was identified 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-hydroxymethyl-6-methyl-6-*O*-[4-*O*-((6*S*)-2-*trans*-2,6-dimethly-β-D-(quinovopyanosyl)-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 and named Julibroside J_{13} (Fig. 2). Compound **4** is a diastereoisomer of **3**.

Julibroside, J_{12} and J_{13} showed marked cytotoxic activities against Bel-7402 cancer cell line at 100 μg/mL assayed by SRB method.

3. Experimental

3.1. 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 KBr disks. 1D and 2D NMR (HSQC, spin time for TOCSY is 100 ms, HMBC-d_6 = 70 ms, NOESY) were recorded using Bruker AM-500 and Varian-300 instruments. MALDI-TOF-MS were taken on BIFLEX III TOF mass spectrometer. FABMS were recorded using a ZABspec mass spectrometer. High-performance liquid chromatography was carried out using Gilson automatic system for preparative HPLC with chromatography col-

umn: Alltima C_{18} (5 μm, 60 Å, 22 × 250 mm ID and 10 μm, 60 Å, 22 × 250 mm ID), using Waters 600 HPLC meter for semi-preparative HPLC with chromatography column: μBondpak C_{18} (6 μm, 60 Å, 7.8 × 300 mm ID). Macroporous resin D_{101} (Nankai University) and HP-20 (Beijing Green Herbs S.T.D. Co. Ltd), silica gel (10–40, 200–300 mesh, Qingdao), Sephadex LH-20 (Pharmacia) and Rp C_{18} silica gel (100–200 mesh, Ouya) were used as normal and reversed phases, respectively, for chromatographic separations.

3.1.1. Plant material. Dried stem bark of *A. julibrissin* was purchased from Mianyang Medicinal Company of Sichuan Province in October 1995, and identified by Professor Shen-hua Li. A sample was deposited in the Department of Natural Medicines, Peking University.

3.2. Extraction and isolation

Air-dried powdered stem bark (13.5 kg) was extracted with 95% ethanol. The ethanol residue (1140 g) was suspended in H_2O and extracted successively with $CHCl_3$, EtOAc and *n*-BuOH. The *n*-BuOH soluble part was dissolved in MeOH, then poured into acetone dropwise. Precipitates were chromatographed over D_{101} macroporous resin column by elution with gradient solvent system (100% H_2O → 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 white powder (22.5 g). The white powder (10.5 g) was subjected to repeated Sephadex LH-20, Rp C_{18} silica gel column chromatography to give seven fractions. And finally sixth fraction was separated by semi-preparative and preparative HPLC to afford **1** (64 mg), **2** (89 mg), **3** (15 mg) and **4** (14 mg).

3.3. Acid hydrolysis of 1

Compound **1** (20 mg) was hydrolyzed by 2 mol/L HCl at 100 °C for 12 h. After filtration of the reaction mixture, the precipitate was identified as acacic acid lactone by silica gel HPTLC ($CHCl_3$–MeOH = 95:5 and C_6H_{12}–Me_2CO = 3:1). The filtrate was neutralized with Ag_2CO_3 to give a residue. The residue was identified as fucose, xylose, glucose, rhamnose, arabinose and quinovose on PC (*n*-BuOH–HOAc–H_2O = 4:1:2).

3.4. Alkaline hydrolysis of 1 and 2

A solution of **1** (12 mg) and **2** (15 mg) in 1% $NaHCO_3$–H_2O was refluxed for 1 h, respectively. After neutralization with 1 M HCl/H_2O, the reaction mixture was fractioned by macroporous HP-20 column, eluting with H_2O and 100% MeOH. The 100% MeOH eluate of alkaline hydrolysis of **1** and **2** was subjected to HPLC on ODS with 18% CH_3CN in H_2O to yield **1B** (2.0 mg) and **2B** (2.2 mg).

3.5. Enzymatic hydrolysis of 1B and 2B

Compound **1B** was hydrolyzed with 2 mL emulsin–NaOH–NaAc (pH = 5) at room temperature for 72 h. The mixture was extracted with $CHCl_3$ to give **1C**.

Compound **2B** was hydrolyzed as described for **1B** to give **2C**.

3.6. Identification

Compound **1**: a white amorphous powder; $[\alpha]_D^{14}$ −35.0 (*c* 0.050, 70% MeOH); RI (KBr) ν_{max} cm^{-1}: 3429, 2928, 1700, 1640, 1561, 1383, 1277, 1072, 638. The MALDI-TOF-MS showed the quasi-molecular ion peak at *m/z* 2193 $[M+Na]^+$. ^1H NMR (Py-d_5): δ 1.30, 1.02, 0.96, 1.15, 1.89, 1.03, 1.07 (each 3H, s, H-23, 24, 25, 26, 27, 29, 30), 3.58 (1H, m, H-3α), 5.20 (1H, m, H-16β), 6.30 (1H, m, H-21α), 5.61 (1H, br s, H-12). ^1H NMR data of the sugars, MT, MT′ and ^{13}C NMR data see Tables 1–3.

Compound **1B**: a white powder; $[\alpha]_D^{14}$ −44.9 (*c* 0.18, MeOH); ^1H NMR (Py-d_5): δ 7.21 (1H, t, *J* = 7.8 Hz, H-3), 6.32 (1H, dd, *J* = 11.1, 17.7 Hz, H-7), 5.33 (1H, d, *J* = 17.7 Hz, H-8b), 5.19 (1H, d, *J* = 10.8 Hz, H-8a), 4.85 (1H, d, *J* = 7.5 Hz, H-qui-1), 2.55 (2H, m, H-4), 2.03 (3H, s, H-9), 1.89 (2H, t, *J* = 8.1 Hz, H-5), 1.60 (3H, d, *J* = 4.8 Hz, H-qui-6), 1.25 (3H, s, H-10). ^{13}C NMR (Py-d_5) (C-1–10): δ 170.3, 128.6, 142.1, 23.3, 38.3, 79.2, 144.1, 113.8, 126, 24.5; (C-qui-1–6): 98.9, 75.1 78.1, 76.6, 72.4, 18.5. It gave a $[M-H]^-$ peak at *m/z* 329 in the negative-ion FAB-MS.

Compound **1C**: $[\alpha]_D^{14}$ −14.6 (*c* 0.52, $CDCl_3$); ^1H NMR δ 6.88 (1H, t, *J* = 7.5 Hz, H-3), 5.89 (1H, dd, *J* = 10.8, 17.4 Hz, H-7), 5.22 (1H, d, *J* = 17.4 Hz, H-8b), 5.08 (1H, d, *J* = 10.8 Hz, H-8a), 2.23 (2H, m, H-4), 1.81 (3H, s, H-9), 1.66 (2H, m, H-5), 1.30 (3H, s, H-10).

Compound **2**: a white amorphous powder; $[\alpha]_D^{14}$ −28.6 (*c* 0.035, 70% MeOH); The MALDI-TOF-MS gave the quasi-molecular ion peak at *m/z* 2194 $[M+H+Na]^+$. Positive FAB-MS at *m/z*: 2195 $[M+Na+2]^+$, 2032 $[M+Na+2\text{-glc}]^+$, 1917 $[M+Na+2\text{-qui-xyl}]^+$, 1765 $[M+Na+2\text{-glc-ara-xyl}]^+$, 1592 $[M+Na+2\text{-(2glc+rha+ara)}]^+$, 1447 $[M+Na+2\text{-(2glc+rha+ara)-qui}]^+$. IR (KBr) ν_{max} cm^{-1}: 3416, 2923, 1692, 1639, 1383, 1277, 1017. ^1H NMR (500Hz, Py-d_5): δ 1.29, 1.03, 0.96, 1.15, 1.87, 1.03, 1.08 (each 3H, s, H-23, 24, 25, 26, 27, 29, 30), 5.60 (1H, br s, H-12); δ 6.29 (1H, m, H- 21), 6.24 (1H, br s, H-ara-1), 6.03 (1H, d, *J* = 7.6 Hz, H-glc′-1), 5.87 (1H, br s, H-rha-1), 5.32 (1H, d, *J* = 7.8 Hz, H-glc″-1), 5.08 (1H, d, *J* = 6.9 Hz, H-xyl-1), 4.99 (1H, d, *J* = 7.5 Hz, H-fuc-1), 4.94 (1H, d, *J* = 8.5 Hz, H-glc-1),4.85 (1H, d, *J* = 7.8 Hz, H-qui′-1), 4.83 (1H, d, *J* = 7.8 Hz, H-qui-1), 1.74 (3H, d, *J* = 6.2 Hz, H-rha-6), 1.58 (3H,

d, $J = 5.2$ Hz, H-qui′-6), 1.46 (3H, d, $J = 7.8$ Hz, H-fuc-6), 1.33 (3H, d, $J = 6.1$ Hz, H = qui-6). For ^{1}H NMR data of MT, MT′ and ^{13}C NMR (125 MHz, Py-d_5) see Tables 1–3.

Compound **2B**: a white powder; $[\alpha]_D^{14}$ −97 (*c* 0.21, MeOH); ^{1}H NMR (Py-d_5): δ 7.17 (1H, t, $J = 7.8$ Hz, H-3), 6.22 (1H, dd, $J = 10.8$, 17.4 Hz, H-7), 5.42 (1H, d, $J = 17.7$ Hz, H-8b), 5.22 (1H, d, $J = 10.8$ Hz, H-8a), 4.88 (1H, d, $J = 7.2$ Hz, H-qui-1), 2.45 (2H, m, H-4), 2.00 (3H, s, H-9),1.80 (2H, t, $J = 8.1$ Hz, H-5), 1.59 (3H, d, $J = 5.1$ Hz, H-qui-6), ^{13}C NMR (75 MHz, Py-d_5) C-1–10: δ 170.3, 128.7, 141.9, 23.5, 40.3, 79.2, 143.8, 114.5, 12.5, 23.5; (C-qui-1–6): 99.0, 75.2, 78.1, 76.6, 72.3, 18.6. The negative FAB mass spectrum showed quasi-molecular ion peak at *m*/*z* 329 $[M-1]^-$.

Compound **2C**: $[\alpha]_D^{14}$ +18.2 (*c* 0.22, $CDCl_3$). ^{1}H NMR (75 Hz, $CDCl_3$): δ 6.89 (1H, t, $J = 7.2$ Hz, H-3), 5.89 (1H, dd, $J = 10.8$, 17.4 Hz, H-7), 5.22 (1H, d, $J =$ 17.4 Hz, H-8b), 5.08 (1H, d, $J = 10.8$ Hz, H-8a), 2.24 (2H, m, H-4), 1.82 (3H, s, H-9), 1.66 (2H, m, H-5), 1.30 (3H, s, H-10).

Compound **3**: a white amorphous powder; $[\alpha]_D^{14}$ +7.7 (*c* 0.078, 70% MeOH); The MALDI-TOF-MS gave the quasi-molecular ion peak at *m*/*z* 2235 $[M+1+Na]^+$. IR(KBr) ν_{max} cm^{-1}: 3406, 2928, 1692, 1640, 1382, 1072, 640. ^{1}H NMR (500 Hz, Py-d_5): δ 1.18, 1.03, 0.97, 0.94, 1.16, 1.87, 1.03, 1.08 (each 3H, s), 5.61 (1H, br s, H-12); 6.26 (1H, br s, H-ara-1), 6.04 (1H, d, $J = 7.5$ Hz, H-glc′-1), 5.88 (1H, br s, H-rha-1), 5.61 (1H, br s, H-12), 5.34 (1H, d, $J = 7.6$ Hz, H-glc″-1), 5.07 (1H, d, $J = 6.6$ Hz, H-xyl-1), 4.97 (1H, d, $J = 7.5$ Hz, H-fuc-1), 4.95 (1H, d, $J = 7.2$ Hz, H-glc-1), 4.82 (1H, d, $J = 7.7$ Hz, H-qui′-1), 4.84 (1H, d, $J = 8.0$ Hz, H-qui-1), 1.75 (3H, d, $J = 5.2$ Hz, H-rha-6), 1.58 (3H, d, $J = 5.1$ Hz, H-qui′-6), 1.47 (3H, d, $J = 6.8$ Hz, H-fuc-6), 1.34 (3H, d, $J = 5.9$ Hz, H-qui-6). 7.02 (1H, br s, MT-H-3), 6.19 (1H, dd, $J = 10.7$,17.9 Hz, MT-H-7), 5.21 (1H, d, $J = 10.7$ Hz, MT-H-8a), 5.40 (1H, d, $J = 17.9$ Hz, MT-H-8b), 4.71 (2H, s, MT-H-9), 1.50 (3H, s, MT-H-10), 7.10 (1H, t, $J = 7.0$ Hz MT′-H-3), 6.31 (1H, dd, $J = 11.1$, 17.6 Hz, MT′-H-7), 5.19 (1H, d, $J = 11.1$ Hz, MT′-H-8a), 5.32 (1H, d, $J = 17.6$ Hz, MT-H-8b), 1.93 (3H, s, MT′-H-9), 1.45 (3H, s, MT′-H-10). For ^{13}C NMR (125 MHz, Py-d_5) see Tables 1–3.

Compound **4** was obtained as a white amorphous powder; $[\alpha]_D^{14}$ +23.8 (*c* 0.11, 70% MeOH); The MALDI-TOF-MS gave the quasi-molecular ion peak at *m*/*z* 2235 $[M+1+Na]^+$. IR (KBr) ν_{max} cm^{-1}: 3388, 2927, 1692, 1637, 1382, 1070. ^{1}H NMR (500 MHz, Py-d_5): δ 1.19, 1.02, 0.96, 0.94, 1.15, 1.86, 1.03, 1.07 (each 3H, s), 5.58 (1H, m, 12-H); 6.26 (1H, br s, H-ara-1), 6.04 (1H, d, $J = 7.8$ Hz, H-glc′-1), 5.88 (1H, br s, H-rha-1), 5.58 (1H, br s, H-12), 5.32 (1H, d, $J = 7.7$ Hz, H-glc″-1), 5.06 (1H, d, $J = 6.7$ Hz, H-xyl-1), 4.98 (1H, d, $J = 7.0$ Hz, H-fuc-1), 4.92 (1H, d, $J = 7.4$ Hz, H-glc-1), 4.86 (1H, d, $J = 7.7$ Hz, H-qui′-1), 4.84 (1H, d, $J = 7.6$ Hz, H-qui-1), 1.76 (3H, d, $J = 5.6$ Hz, H-rha-6), 1.58 (3H, d, $J = 5.2$ Hz, H-qui′-6), 1.46 (3H, d, $J = 6.1$ Hz, H-fuc-6), 1.33 (3H, d, $J = 6.0$ Hz, H = qui-6), 7.02 (1H, br s, MT-H-3), 6.19 (1H, dd, $J = 11.0$, 17.5 Hz, MT-H-7), 5.21 (1H, d, $J = 11.0$ Hz, MT-H-8a), 5.39 (1H, d, $J = 17.5$ Hz, MT-H-8b), 4.70 (2H, s, MT-H-9), 1.50 (3H, s, MT-H-10), 6.96 (1H, br s, MT′-H-3), 1.71 (2H, t, $J = 7.6$ Hz, MT′-H-5), 6.19 (1H, dd, $J = 11.0$, 17.5 Hz, MT-H-7), 5.21 (1H, d, $J = 11.0$ Hz, MT′-H-8a), 5.41 (1H, d, $J = 17.5$ Hz, MT′-H-8b), 1.86 (3H, s, MT′-H-9), 1.53 (3H, s, MT′-H-10). For ^{13}C NMR (125 MHz, Py-d_5) see Tables 1–3.

Table 5. The Inhibition (%) against Bel-7402 of compounds **1**–**4**

Compound	Concentration (μg/mL)		
	(1.0)	(10.0)	(100.0)
Julibroside J_5 (**1**)	−17.30	52.43	58.29
Julibroside J_8 (**2**)	39.75	58.32	86.66
Julibroside J_{12} (**3**)	13.53	40.99	63.98
Julibroside J_{13} (**4**)	29.69	56.37	93.33

3.7. Cytotoxicity bioassays against cancer cells

The MTT and SRB method were used for the assay of Cytotoxicity in vitro to HL-60 (MTT method), BGC-823 (SRB), Bel-7402 (SRB), and Hela (SRB) cancer cell lines at 1.0, 10.0 and 100.0 μg/mL. Julibroside J_8 and J_{13} showed marked cytotoxic activities against Bel-7402 cancer cell line at 100 μg/mL assayed by SRB method (see Table 5).

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