Three New Triterpenoidal Saponins Acylated with Monoterpenic Acid from Albizziae Cortex

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The structures of Julibrosides I, II, and III, isolated from dried stem bark of *Albizzia julibrissin* DURAZZ (Albizziae Cortex), were characterized as complex triterpenoidal glycosides linked to the monoterpene glycoside dimer, mainly by means of an NMR spectroscopic method. These compounds have a common structure comprising from acacic acid, the (6*S*)-2-*trans*-6-hydroxy-2,6-dimethyl-2,7-octadienoyl-6-*O*-quinovopyranoside dimer, and α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. The diversity of the oligosaccharides attached to the C-3 hydroxyl group of acacic acid, an oleanane-type triterpene, makes a distinction between Julibrosides I, II, and III.

The dried stem bark of *Albizzia julibrissin* DURAZZ, Albizziae Cortex, is used as a tonic in China and Japan. During our study of the constituents of leguminous plants, we isolated the minor components of six new triterpenoidal glycosides, including Julibroside A_1, A_2, and A_3 (**1a**, **2a**, and **3a**) (Fig. 1), from this crude drug.[1] On the other hand, a Korean group reported that triterpene sapogenols acylated with monoterpenic acid obtained by acid hydrolysis from the same crude drug.[2] However, there has been no report on the more polar saponins as the main components. This paper deals with the isolation and structural elucidation of three major saponins from this crude drug.

The plant was extracted with MeOH, and the extract was partitioned with 1-butanol and water. The aqueous extract was chromatographed on various reversed-phased columns (MCI gel CHP-20P, Bondpak C_{18}, Wako gel LP60 C_{18}, Chromatorex ODS), and a silica-gel column to give three triterpenoidal saponins called Julibroside I (**1**), II (**2**), and III (**3**) (Fig. 2).

Julibroside I (**1**) was obtaind as a white amorphous powder, $[\alpha]_D$ −26.4° (MeOH). The infrared (IR) spectrum of **1** featured absorptions of the carbonyl group (1695 cm^{-1}) and α,β-unsaturated carbonyl group (1645 cm^{-1}). In the positive fast atom bombardment-mass spectrum (FAB-MS), **1** showed an $[M+Na]^+$ ion at *m/z* 2340 (uncorrected), and an $[M-H]^-$ ion at *m/z* 2316 (uncorrected) in the negative FAB-MS. The high-resolution FAB-MS gave an $[M-H]^-$ ion at

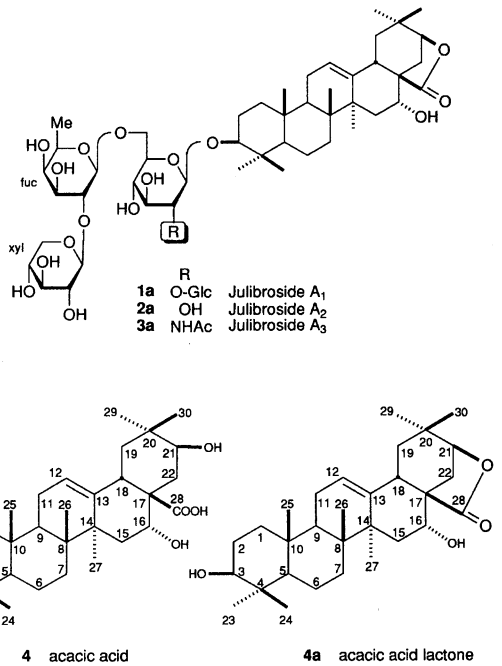

Fig. 1. The structures of Julibroside A_1 (**1a**), Julibroside A_2 (**2a**), Julibroside A_3 (**3a**), acacic acid (**4**), and acacic acid lactone (**4a**).

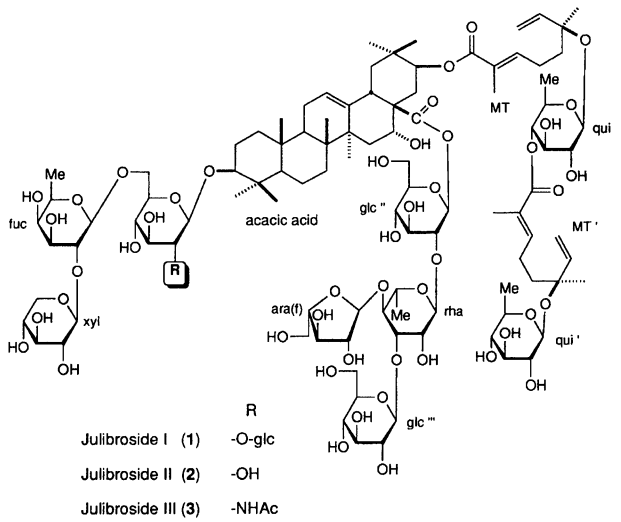

Fig. 2. The structures of Julibroside I (**1**), Julibroside II (**2**), Julibroside III (**3**); glc: glucopyranose, fuc: fucopyranose, xyl: xylopyranose, rha: rhamnopyranose, ara (f): arabinofuranose, qui: quinovopyranose, MT: (6*S*)-2-*trans*-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid.

m/z 2317.0569, which corresponds to the composition $^{12}C_{107}{}^{13}CH_{171}O_{53}$. Upon acid hydrolysis with 2 M hydrochloric acid (1 M=1 mol dm^{-3}), **1** gave an acacic acid lactone, which was identified with an authentic sample,[1] arabinose, fucose, glucose, quinovose, rhamnose, and xylose, as component sugars. The proton and carbon-13 nuclear magnetic resonance (NMR) signals of **1** taken in pyridine-d_5 solution were assingned by proton–proton correlation spectroscopy (^{1}H–^{1}H COSY), nuclear Overhauser effect spectroscopy (NOESY), and Relayed COSY, followed by carbon-13-proton correlation spectroscopy (^{13}C–^{1}H COSY).

The ^{1}H NMR spectrum showed signals ascribable to eleven tertiary methyl groups (δ=0.99, 1.01, 1.08, 1.17, 1.18, 1.29, 1.55, 1.56, 1.85, 1.90×2) and nine olefinic protons [δ=7.05 (1H, dt, J=1.5, 7.6 Hz), 6.91 (1H, dt, J=1.5, 7.6 Hz), 6.24 (1H, dd, J=11.0, 17.7 Hz), 6.23 (1H, dd, J=10.9, 17.6 Hz), 5.63 (1H, br s), 5.47 (1H, dd, J=1.4, 17.6 Hz), 5.44 (1H, dd, J=1.5, 17.7 Hz), 5.29 (1H, dd, J=1.2, 10.9 Hz), 5.24 (1H, dd, J=1.4, 11.0 Hz)]. These observation suggested that **1** was an triterpenoidal saponin which was acylated with some esters. The signals of ten anomeric protons [δ=6.26 (1H, d, J=8.4 Hz), 6.07 (1H, d, J=8.0 Hz), 5.90 (1H, br s), 5.43 (1H, d, J=7.6 Hz), 5.34 (1H, d, J=7.7 Hz), 5.07 (1H, d, J=6.9 Hz), 4.98 (1H, d, J=7.7 Hz), 4.89 (1H, d, J=8.3 Hz), 4.88 (2H, d, J=7.7 Hz)], and four secondary methyl signals ascribable to 6-deoxyhexose [δ=1.36 (3H, d, J=6.2 Hz), 1.51 (3H, d, J=6.4 Hz), 1.60 (3H, d, J=5.7 Hz), 1.80 (3H, d, J=6.0 Hz)] indicated that **1** had ten sugars including four 6-deoxyhexoses. The ^{13}C NMR spectrum of **1** (Tables 1, 2, 3, and 4) showed ten anomeric carbons (δ=111.07, 107.03, 105.85, 105.76, 104.91, 103.45, 101.86, 99.36×2, 95.71),

Table 1. ^{13}C NMR Spectral Data in Pyridine-d_5 (Triterpene Part)

	1	**2**	**3**	**4**	**5**	**6**
C-1	40.0	38.9	38.7	39.0	38.7	39.0
2	26.9	26.8	26.5	28.1	26.6	26.6
3	88.6	88.8	88.8	78.1	88.5	90.7
4	39.7	39.5	39.3	39.4	39.5	39.9
5	56.1	56.0	55.9	56.0	55.9	56.3
6	18.7	18.7	18.6	18.9	18.5	18.6
7	33.7	33.6	33.6	33.6	33.4	33.7
8	40.2	40.1	40.1	39.9	39.9	39.9
9	47.2	47.1	47.1	47.3	46.9	47.5
10	37.1	37.0	37.0	37.5	36.9	36.5
11	24.0	23.9	23.8	23.9	23.7	24.0
12	123.1	123.1	123.0	122.7	122.9	122.3
13	143.4	143.7	143.2	144.5	143.2	145.2
14	42.1	41.9	41.9	42.1	41.8	42.0
15	35.9	35.6	35.8	35.9	35.7	35.4
16	73.9	73.6	73.8	74.4	73.7	75.3
17	51.7	51.7	51.6	51.8	51.5	53.4
18	41.0	40.8	40.9	41.1	40.8	41.5
19	47.9	47.7	47.7	48.6	47.7	49.2
20	35.3	35.2	35.2	36.7	35.1	37.1
21	77.1	76.5	76.8	73.5	76.7	74.4
22	36.5	36.2	36.3	41.9	36.2	40.9
23	28.2	28.2	28.1	28.8	27.9	28.1
24	15.9	15.8	15.7	15.7	15.7	15.8
25	16.9	17.0	17.0	16.6	16.7	16.8
26	17.4	17.3	17.2	17.6	17.0	17.8
27	27.3	27.2	27.2	27.2	27.1	27.3
28	174.5	174.8	174.4	179.4	174.3	182.3
29	29.2	29.0	29.1	30.1	29.0	30.1
30	19.2	19.1	19.0	18.4	19.0	18.6

one ester carbonyl carbon (δ=174.50), two α,β-unsaturated ester carbonyl carbons (δ=167.80, 167.78), one pair of the olefinic carbon of the aglycone part [δ=123.11 (d), 143.41 (s)], two pairs of trisubstituted olefinic carbons [δ=143.41 (d), 128.57 (s), 142.32 (d), 127.98 (s)], two pairs of monosubstituted olefinic carbons [δ=144.11 (d), 114.85 (t), 144.01 (d), 115.12 (t)], and one sugar-linked methine carbon (δ=88.62). These data supported that **1** was an acacic acid (**4**) decaglycosides acylated with two monoterpenic acids. However, a comparative study of the ^{13}C NMR data of **1** and **4** led to the identification of glycosylation shifts[3] at C-2 (−1.2 ppm) and C-3 (+10.5 ppm), and acylation shifts[4] at C-20 (−1.4 ppm), C-21 (+3.6 ppm), and C-22 (−5.4 ppm). Furthermore, an upfield C-28 signal was observed in **1** (δ=174.50), compared with **4** (δ=179.4). The above facts suggest that **1** should be a 3,21,28-*O*-trisdesmoside having decaglycosides of **4** along with two monoterpenic acids.

Upon the alkaline hydrolysis of the crude saponin fraction (Chart 1) with saturated $NaHCO_3$ in MeOH, a prosapogenin (**5**) was obtained as the major component. A partial hydrolysis of **1** under the same conditions also gave **5**, which showed an $[M-H]^-$ ion at *m/z* 2003 (corrected) in the FAB-MS. The alkaline

Table 2. ^{13}C NMR Spectral Data in Pyridine-d_5 (Sugar Part at C-3)

C-3	1	2	3	5	6
glc-1	104.9	106.4		104.7	103.8
2	83.0	75.0		82.6	80.9
3	78.0	77.8		76.8	76.9
4	71.4	71.9		71.5	70.4
5	77.6	77.3		77.4	77.1
6	69.2	69.6		69.6	69.2
glcNAc-1			104.6		
2			57.8		
3			75.8		
4			72.1		
5			77.4		
6			69.8		
CH3			23.7		
CO			170.1		
glc′-1	105.9			105.5	104.7
2	75.6			75.7	75.8
3	78.1			78.0	77.6
4	71.8			71.1	70.4
5	78.2			78.0	77.7
6	62.8			62.3	62.5
fuc-1	103.5	103.1	103.3	103.2	102.9
2	82.3	81.8	82.1	82.0	80.2
3	75.2	74.8	75.1	75.2	75.3
4	72.2	72.3	72.4	72.4	72.1
5	71.4	71.2	71.7	71.0	71.4
6	17.3	17.1	17.1	17.2	17.8
xyl-1	107.3	106.4	106.8	106.8	105.6
2	75.9	75.6	75.8	76.3	75.8
3	78.5	77.8	78.3	77.8	77.7
4	70.8	70.4	70.7	70.6	70.2
5	67.2	66.9	67.0	67.0	66.6

glc: inner glucopyranose, glcNAc: 2-acetoamido-2-deoxy-glucopyranose, glc′: terminal glucopyranose, fuc: fucopyranose, xyl: xylopyranose.

Table 3. ^{13}C NMR Spectral Data in Pyridine-d_5 (Sugar Part at C-28)

C-28	1	2	3	5
glc″-1	95.7	95.3	95.5	95.5
2	77.6	76.3	76.7	78.9
3	78.0	77.6	78.0	76.8
4	71.3	70.8	71.1	72.0
5	79.1	78.4	78.9	78.2
6	62.0	62.3	61.9	62.6
rha-1	101.9	101.7	101.7	101.7
2	70.6	70.4	70.5	70.3
3	82.0	81.7	81.9	81.8
4	79.1	78.8	79.0	78.8
5	69.2	69.1	69.0	69.0
6	18.9	18.8	18.7	18.7
glc‴-1	105.8	105.4	105.7	105.5
2	75.4	75.1	75.3	75.0
3	78.5	77.8	78.3	77.9
4	71.4	71.2	71.2	71.1
5	78.3	77.8	78.1	78.2
6	62.0	61.8	62.4	62.6
ara (f)-1	111.1	110.5	110.9	110.8
2	84.5	83.8	84.3	84.2
3	78.4	78.0	78.3	78.2
4	85.5	85.1	85.4	85.2
5	62.6	62.3	62.6	61.8

glc″: inner glucopyranose, rha: rhamnopyranose, glc‴: terminal glucopyranose, ara (f): arabinofuranose.

hydrolysis of **5** with 3% KOH in MeOH and purified by MCI gel gave a prosapogenin (**6**) and monoterpene glycoside (**7**) as major products. Furthermore, **6** was converted to Julibroside A_1 (**1a**) by a cation-exchange resin (Amberlite® IR-120B).[1] Therefore, **6** was concluded to be 3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)]-β-D-glucopyranosyl} acacic acid. Compound **7** was hydrolyzed with β-glucosidase to afford a monoterpenic acid (**8**), which was identified with an authentic sample of (6*S*)-2-*trans*-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid.[5] The ^{1}H NMR spectrum of **7** showed the presence of an anomeric proton at δ=4.99 (d, J=7.7 Hz) and a secondary methyl group at δ=1.62 (d, J=5.5 Hz) related to 6-deoxyhexose. The ^{13}C NMR spectrum of **7** (Table 4) showed the presence of an anomeric carbon at δ=99.3 and five signals at δ=75.4, 78.3, 76.8, 72.5, and 18.3, indicating the presence of the β-D-quinovopyranosyl unit. Since a glycosylation shift was observed at the C-6 (+6.3 ppm) in **7**, the structure of **7** was established to be (6*S*)-2-*trans*-2,6-dimethyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoic acid. In the ^{13}C NMR spectrum of **5** (Table 1), since the signals for the sapogenol part were in good agreement with those of acaciaside A,[6] **7** was concluded to attach at C-21.

Meanwhile, in the FAB-MS of **1**, since a major fragment ion peak was observed at m/z 1737 $[M+Na-602]^+$, the sugar moiety linked to the C-28 was deduced to have the mass number 602.[7] Since this fragment ion peak was also observed in the case of **5**, the sugar moiety at C-28 of **1** had four sugars consisting of two hexoses, one 6-deoxyhexose and one pentose. In the ^{13}C–^{1}H COSY of **1**, the anomeric carbon at δ=95.71 was correlated with the anomeric proton δ=6.07. Starting from this anomeric proton, the assingments of the inner sugar protons (H-2—H-6) at C-28 were determined by ^{1}H–^{1}H COSY and Relayed COSY. In the NOESY spectrum, the H-2 proton (δ=4.01) of inner sugar was correlated to the anomeric proton (H′-1, δ=5.90, br s) (Fig. 3). The respective protons of the sugar moiety attached to C-2 were assinged in a similar manner to H′-1 (δ=5.90), H′-2 (δ=5.17), H′-3 (δ=4.94), H′-4 (δ=4.52), H′-5 (δ=4.56), and H′-6 (δ=1.80). In addition, the cross peaks between H′-3 (δ=4.94) and the anomeric proton (H″-1, δ=5.34, 1H, d, J=7.7 Hz), and H′-4 (δ=4.52) and

Table 4. ^{13}C NMR Spectrum Data in Pyridine-d_5 (Monoterpene Derivaties)

	1	2	3	5	7	8
MT-1	167.8	168.2	167.7	167.6	171.3	173.2
2	128.0	127.8	128.4	128.2	122.9	127.3
3	142.3	142.9	142.2	142.3	142.0	144.4
4	23.6	23.7	23.5	23.45	23.66	23.70
5	40.5	40.2	40.3	40.5	40.6	40.5
6	79.5	79.7	79.4	79.3	79.5	73.2
7	144.0	143.8	143.9	143.9	144.1	144.6
8	115.1	115.5	114.7	114.7	114.7	112.3
9	12.7	12.6	12.6	12.5	12.8	12.0
10	23.7	23.5	23.5	23.54	23.69	28.00
qui-1	99.4	99.1	99.2	99.1	99.3	
2	75.7	75.5	75.5	75.3	75.4	
3	75.7	75.4	75.5	78.2	78.3	
4	77.2	77.1	77.1	76.7	76.8	
5	70.2	70.1	70.1	72.4	72.5	
6	18.5	18.3	18.3	18.7	18.8	
MT′-1	167.8	168.2	167.7			
2	128.6	128.3	129.0			
3	143.4	143.6	143.6			
4	23.8	23.7	23.6			
5	40.4	40.4	40.4			
6	79.8	80.0	79.7			
7	141.1	143.8	144.0			
8	114.9	115.2	115.0			
9	12.7	12.6	12.6			
10	24.0	23.6	23.6			
qui′-1	99.4	99.0	99.1			
2	75.6	75.1	75.5			
3	78.5	78.0	78.3			
4	77.1	77.0	77.0			
5	72.6	72.3	72.5			
6	18.9	18.3	18.6			

MT: (6*S*)-2-*trans*-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid, qui: quinovopyranose.

the anomeric proton (H‴-1, δ=6.26, 1H, d, J=8.4 Hz) were observed in the NOESY spectrum (Fig. 3). In the same manner, each protons attached at the second saccharide was assigned to H″-1 (δ=5.34), H″-2 (δ=4.01), H″-3 (δ=4.17), H″-4 (δ=4.10), H″-5 (δ=3.93), H″-6 (δ=4.22, 4.51) and H‴-1 (δ=6.26), H‴-2 (δ=5.00), H‴-3 (δ=4.82), H‴-4 (δ=4.76), and H‴-5 (δ=4.16, 4.27). Since the respective carbon signals were assigned by the ^{13}C–^{1}H COSY (Fig. 4), the structure of sugar moiety at C-28 was determined to be α-L-arabinofuranosyl-(1→4)−[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. Thus, the structure of **5** was concluded to be 21-*O*-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)]-β-D-glucopyranosyl} acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

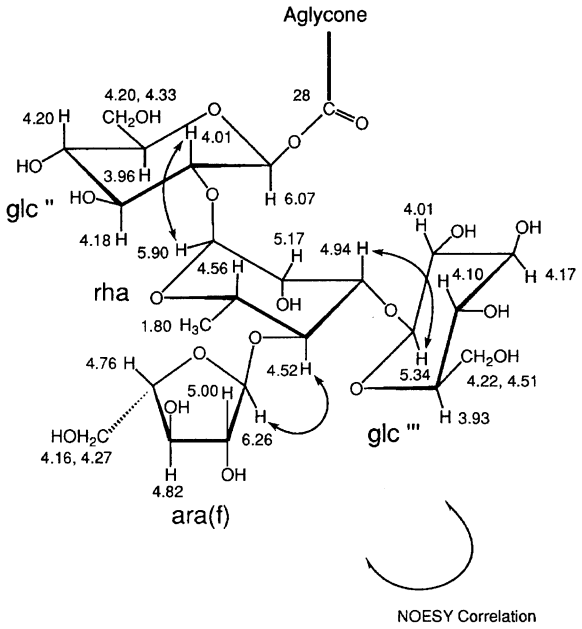

Fig. 3. NOESY experiment of sugar moiety at C-28 of **1**.

On the other hand, when the FAB-MS data of **1** and **5** were compared, **1** showed an additional 6-deoxyhexosyl monoterpene unit. The terminal monoterpene glycoside unit was identical with **7** in the ^{13}C NMR spectrum of **1** (Table 4). Based on the ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY of **1**, the H-4 proton of the inner quinovose (δ=5.36) was shifted to downfield more than that of **7** (ca. 1 ppm); the C-3 and C-5 signals for the quinovopyranosyl unit were also shifted upfield by acylation. Therefore, the ester residue at C-21 in **1** was determinded to be 21-*O*-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-(4-*O*-(6*S*′)-2′-*trans*-2′,6′-dimethyl-6′-*O*-β-D-quinovopyranosyl-2′,7′-octadienoyl-β-D-quinovopyranosyl)-2,7-octadienoyl] ester.

Consequently, the structure of Julibroside I (**1**) was characterized as 21-*O*-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-(4-*O*-(6S′)-2′-*trans*-2′,6′-dimethyl-6′-*O*-β-D-quinovopyranosyl-2′,7′-octadienoyl-β-D-quinovopyranosyl)-2,7-octadienoyl]-3-*O*-{β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)]-β-D-glucopyranosyl} acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Julibroside II (**2**) was obtaind as a white amorphous powder, $[\alpha]_D$ −27.7° (MeOH). In the positive FAB-MS, **2** showed an $[M+Na]^+$ ion at m/z 2178; the negative FAB-MS data were consistent with an $[M-H]^-$ ion at m/z 2154 (uncorrected) and $[M-H-602]^-$ ion at m/z 1552. The high-resolution FAB-MS gave an $[M+Na]^+$ ion at m/z 2179.0342, which corresponds to the composition $^{12}C_{101}{}^{13}CH_{162}O_{48}Na$. The alkaline hydrolysis of **2** with 3% KOH in MeOH and neutralized with cation-exchange resin (Amberlite® IR-120B) gave Julibroside A_2 (**2a**)[1] and **7** as major products. By com-

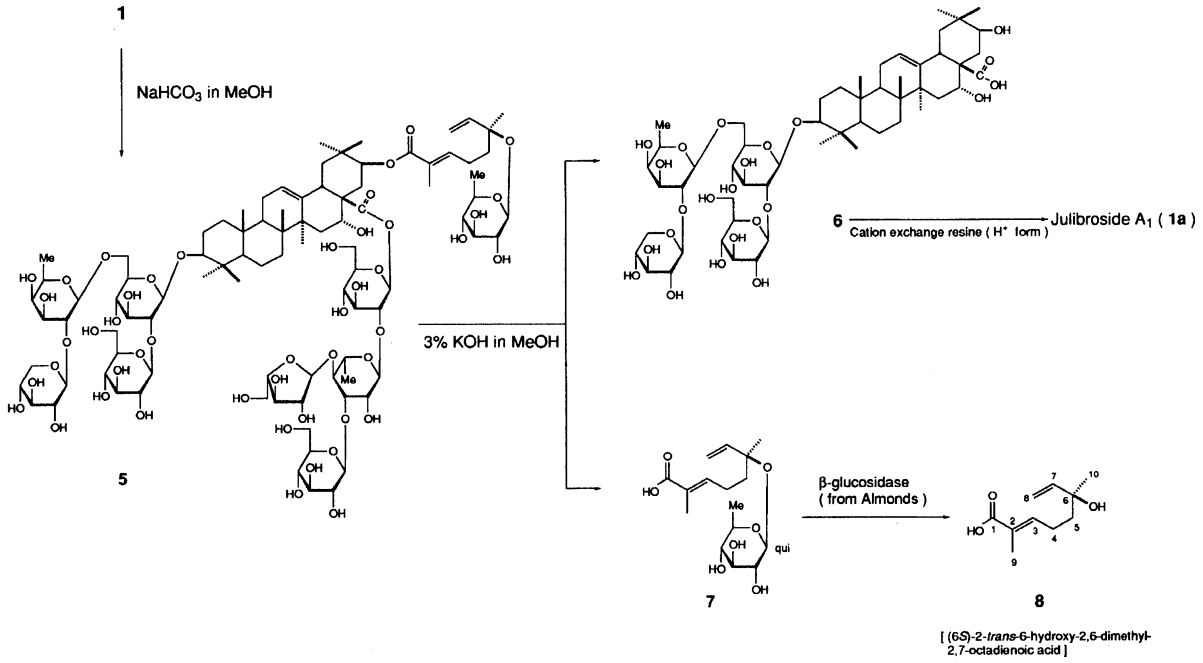

Chart 1.

paring the FAB-MS of **2** with that of **1**, the molecular ion peak of **2** was 162 mass units smaller than that of **1**. This showed that **2** was the deshexosyl compound for **1**. A similar fragment ion peak at $[M-H-602]^-$ suggested that the sugar moiety of C-28 was equal to that of **1**. When the ^1H and ^{13}C NMR data of **2** (Tables 1, 2, 3, and 4) were compared with those of **1**, they were in good agreement with **1**, except for the inner glucosyl unit of the sugar moiety at C-3. Therefore, the structure of Julibroside II (**2**) was deduced to be 21-*O*-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-(4-*O*-(6*S'*)-2'-*trans*-2', 6'-dimethyl-6'-*O*-*β*-D-quinovopyranosyl-2',7'-octadienoyl-*β*-D-quinovopyranosyl)-2,7-octadienoyl]-3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosyl] acacic acid 28-*O*-*α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

Julibroside III (**3**) was obtaind as a white amorphous powder, $[\alpha]_D$ −16.4° (MeOH). The alkaline hydrolysis of **3** under the same conditions described above gave Julibroside A_3 (**3a**)[1] and **7** as major products. In the positive FAB-MS, **3** showed an $[M+Na]^+$ ion at *m/z* 2219 (uncorrected); the negative FAB-MS data were consistent with an $[M-H]^-$ ion at *m/z* 2195 (uncorrected) and $[M-H-602]^-$ ion at *m/z* 1593 (uncorrected). The high-resolution FAB-MS gave an $[M-H]^-$ ion at *m/z* 2196.0466, which corresponds to the composition $^{12}C_{103}{}^{13}CH_{164}NO_{48}$. The fragment ion peak of **3** at $[M-H-602]^-$ suggested that the sugar part at C-28 of aglycone was the same as those of **1** and **2**. When the ^1H and ^{13}C NMR data of **3** were compared with those of **2**, they were in good agreement with **2**, except for the sugar moiety at C-3 (Tables 1, 2, 3, and 4). Since the sugar moiety at C-3 was superimposible on those of **3a**, the structure of Julibroside III (**3**) was deduced to be 21-*O*-[(6*S*)-2-*trans*-2,6-dimethyl-6-*O*-(4-*O*-(6*S'*)-2'-*trans*-2',6'-dimethyl-6'-*O*-*β*-D-quinovopyranosyl-2',7'-octadienoyl-*β*-D-quinovopyranosyl)-2,7-octadienoyl]-3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-*β*-D-glucopyranosyl] acacic acid 28-*O*-*α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

This report presents the first isolation and structural characterization of major saponins as a homogeneous state from Albizziae Cortex.

Experimental

The fresh stem bark of *Albizzia julibrissin* DURAZZ was collected in the Botanical Garden of Kumamoto University. The optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded with a JEOL FT-IR, JIR- 6500W. ^1H and ^{13}C NMR spectra were measured with a JEOL JNM-GX 400 and/or GSX 500 FT-NMR spectrometer and the chemical shifts were given on a *δ* (ppm) scale with tetramethylsilane used as an internal standerd. The FAB-MS were measured with a JEOL DX-300 and/or SX102A spectrometer. The HR FAB-MS were measured with a JEOL DX-303 HF spectrometer and taken in a triethylene glycol and *m*-nitrobenzyl alchohol matrix. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). Column chromatography was carried out on Kieselgel 60 (70—230 mesh and 230—400 mesh), MCI gel CHP-20P (Mitsubishi Chemical, Ind.), Bondapak C_{18} (Waters), Wako gel LP60 C18 (Wako Pure Chemical. Ind.), Sephadex LH-20 (Pharmacia), Amberlite IR-120B (Organo), and Chromatorex ODS-DU 3050MT (Fuji Silysia). Analytical HPLC was carried out on a column of Wakosil-II 5C18 (4.6 mm×250 mm). Preparative HPLC was carried out on a column of Nova-pak HR C_{18} (40 mm×100 mm). *β*-Glu-

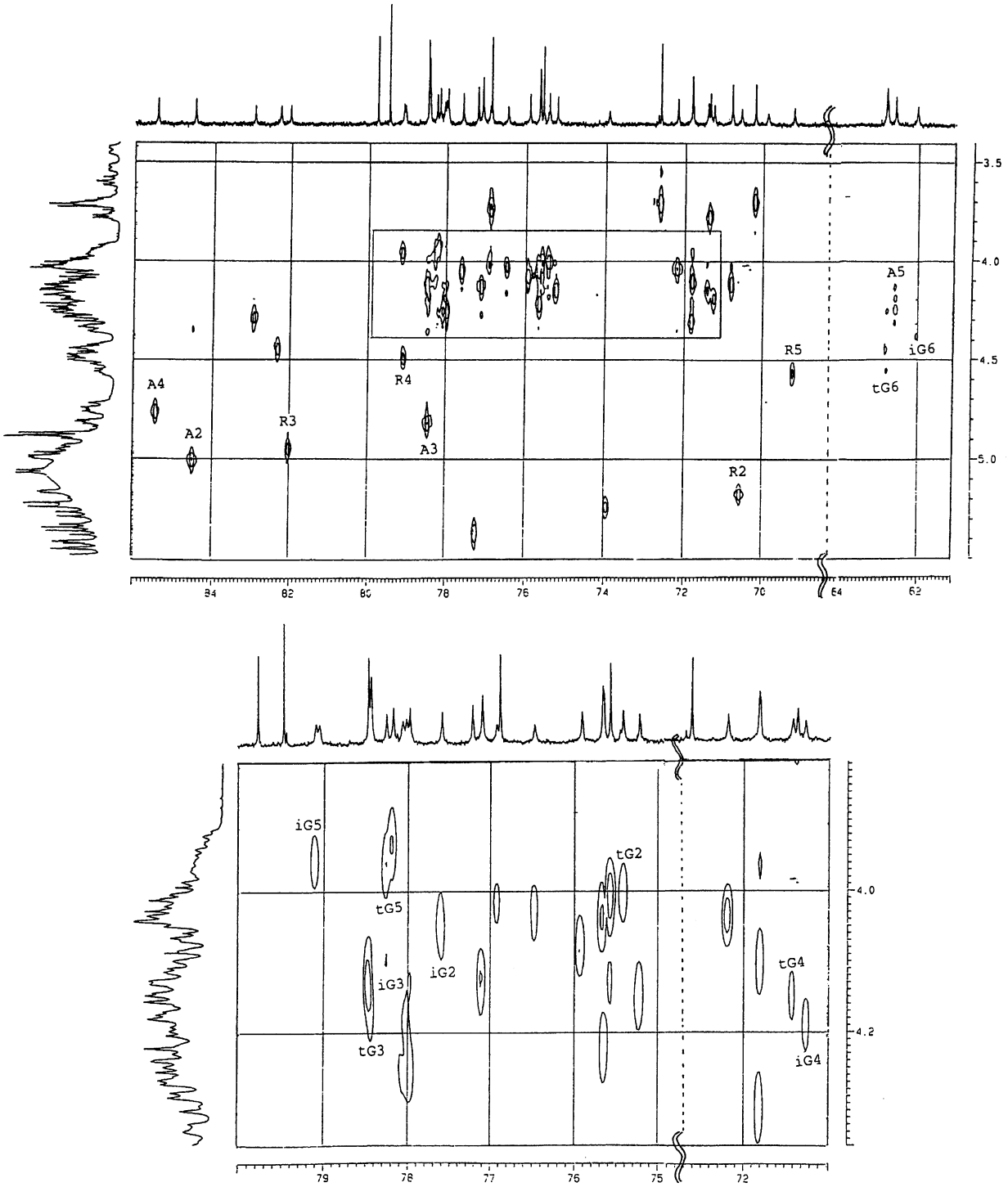

Fig. 4. a) ^{13}C–^{1}H COSY experiment of **1**. The marks of iG, tG, A, and R mean inner glucopyranose, terminal glucopyranose, arabinofuranose, and rhamnopyranose, respectively. The number attaching the marks are corresponded to the posision of sugar part. b) ^{13}C–^{1}H COSY experiment of **1** in the square area of Fig. 4a.

cosidase from Almonds was purchased from Sigma Chemical Co. (St. Louis, USA).

Extraction and Isolation. Fresh stem bark of *Albizzia julibrissin* DURAZZ (6.3 kg) was extracted with MeOH twice under reflux. The combined extract (379 g) was concentrated and partitioned with 1-BuOH and H_2O. The aqueous extract (294 g) was subjected to MCI gel CHP-20P column chromatography using 50→100% MeOH to give fractions 1 to 5. Fraction 2 was separated by Wako gel LP60 C18 (0→100% MeOH), Sephadex LH-20 (0→100% MeOH), silica-gel [$CHCl_3$–MeOH–acetone–acetic acid (3 : 4 : 3 : 1), $CHCl_3$–MeOH–H_2O (26 : 14 : 3)], and Chromatorex ODS-DU 3050MT (60→70% MeOH, 68% MeOH) to provide Julibroside I (**1**) (20 mg). Fraction 3 was separated by

silica-gel [$CHCl_3$–MeOH–H_2O (26:14:3)], Bondapak C_{18} (20→100% MeOH), and preparative HPLC on Nova-pak HR C_{18} (40 mm×100 mm) to yield Julibroside II (**2**) (22 mg). Fraction 4 was separated by silica-gel [$CHCl_3$–MeOH–H_2O (26:14:3)], Bondapak C_{18} (60→70% MeOH) and Chromatorex ODS-DU 3050MT (60→70% MeOH) to yield Julibroside III (**3**) (24 mg).

Julibroside I (1). A white amorphous powder, $[\alpha]_D$ −26.4° (c=0.61, MeOH). IR (KBr) 3396 (ν_{O-H}), 1695, 1645 cm^{-1} ($\nu_{C=O}$). Positive FAB-MS (uncorrected) m/z 2340 $[M+Na]^+$, 2178 $[M+Na-162]^+$, 1866 $[M+Na-146-166-162]^+$, 1738 $[M+Na-602]^+$, negative FAB-MS (uncorrected) m/z 2316 $[M-H]^-$, 1714 $[M-H-602]^-$. HR negative FAB-MS m/z 2317.0569 $[M-H]^-$ ($^{12}C_{107}{}^{13}CH_{171}O_{53}$, Calcd for M, 2317.0719). ^1H NMR (pyridine-d_5) δ=0.99, 1.01, 1.08, 1.17, 1.18, 1.29, 1.55, 1.56, 1.85, 1.90 (2×CH_3) [tertiary methyl groups]; 7.05 (1H, dt, J=1.5, 7.6 Hz), 6.91 (1H, dt, J=1.5, 7.6 Hz), 6.24 (1H, dd, J=11.0, 17.7 Hz), 6.23 (1H, dd, J=10.9, 17.6 Hz), 5.63 (1H, br s), 5.47 (1H, dd, J=1.4, 17.6 Hz), 5.44 (1H, dd, J=1.5, 17.7 Hz), 5.29 (1H, dd, J=1.2, 10.9 Hz), 5.24 (1H, dd, J=1.4, 11.0 Hz) [olefinic protons]; 6.26 (1H, d, J=8.4 Hz), 6.07 (1H, d, J=8.0 Hz), 5.90 (1H, br s), 5.43 (1H, d, J=7.6 Hz), 5.34 (1H, d, J=7.7 Hz), 5.07 (1H, d, J=6.9 Hz), 4.98 (1H, d, J=7.7 Hz), 4.89 (1H, d, J=8.3 Hz), 4.88 (2H, d, J=7.7 Hz) [anomeric protons]; 1.36 (3H, d, J=6.2 Hz), 1.51 (3H, d, J=6.4 Hz), 1.60 (3H, d, J=5.7 Hz), 1.80 (3H, d, J=6.0 Hz) [secondary methyl protons]. ^{13}C NMR (pyridine-d_5): Tables 1, 2, 3, and 4.

Characterization of Sapogenol and Sugars for 1. The glycoside of **1** (5 mg) was dissolved in 2 M HCl/H_2O (2 ml) and heated at 100 °C for 6 h. After filtration of the mixture, the precipitate was identified with acacic acid lactone (**4a**) by TLC [R_f 0.26 ($CHCl_3$–MeOH=10:1), 0.37 (Hexane–Acetone=1:1)],[1,2] while the filtrate was evaporated under an N_2 stream. After the addition of water, the acidic solution was evaporated to again remove HCl. This procedure was repeated until a neutral solution was obtained, which was, finally, evaporated and dried in vacuo. The sugar mixture was subjected to TLC analysis [2-Propanol–H_2O, 9:1, R_f 0.52 (arabinose), 0.60 (fucose), 0.50 (glucose), 0.70 (quinovose), 0.73 (rhamnose), 0.66 (xylose); n-Propanol–Acetone–H_2O, 5:3:1, R_f 0.58 (arabinose), 0.65 (fucose), 0.56 (glucose), 0.73 (quinovose), 0.78 (rhamnose), 0.69 (xylose); Reagent: 20% H_2SO_4].

Mild Alkaline Hydrolysis of Saponin Fraction. Fraction 2 eluted from MCI gel CHP-20P (4 g) in saturated $NaHCO_3$ in MeOH (400 ml) was refluxed for 1 h. The reaction mixture was evaporated to dryness, and separated by a silica-gel column to afford a prosapogenin (**5**) (104 mg). A white amorphous powder, $[\alpha]_D$ −10.9° (c=0.49, MeOH). Positive FAB-MS m/z 2027 $[M+Na]^+$, 1425 $[M+Na-602]^+$, negative FAB-MS m/z 2003 $[M-H]^-$, 1401 $[M-H-602]^-$. HR positive FAB-MS m/z 2028.9121 $[M+Na]^+$ ($^{12}C_{91}{}^{13}CH_{148}O_{47}Na$, Calcd for M, 2028.9122). ^1H NMR (pyridine-d_5) δ=0.99, 1.00, 1.08, 1.16, 1.18, 1.28, 1.56, 1.85, 1.89 [tertiary methyl groups]; 6.96 (1H, t, J=7.0 Hz), 6.21 (1H, dd, J=10.6, 17.6 Hz), 5.63 (1H, br s), 5.45 (1H, d, J=17.6 Hz), 5.24 (1H, d, J=10.6 Hz) [olefinic protons]; 6.23 (1H, d, J=6.2 Hz), 6.06 (1H, d, J=7.7 Hz), 5.90 (1H, br s), 5.42 (1H, d, J=7.0 Hz), 5.33 (1H, d, J=7.7 Hz), 5.06 (1H, d, J=6.2 Hz), 4.97 (1H, d, J=8.1 Hz), 4.95 (1H, d, J=8.1 Hz), 4.87 (1H, d, J=7.3 Hz) [anomeric protons]; 1.49 (3H, d, J=6.2 Hz), 1.60 (3H, d, J=4.3 Hz), 1.78 (3H, d, J=5.9 Hz) [secondary methyl signals]. ^{13}C NMR (pyridine-d_5): Tables 1, 2, 3, and 4.

Mild Alkaline Hydrolysis of 1. A part of **1** (2 mg) in saturated $NaHCO_3$ in MeOH (1 ml) was refluxed for 1h. The reaction mixture was evaporated to dryness, and separated by MCI gel to afford a white amorphous power (1 mg). This compound was identified to be compound **5** by TLC. R_f 0.20 ($CHCl_3$–MeOH–H_2O=6:4:1), HPLC [Wakosil-II 5C18 (4.6 mm×250 mm); mobile phase 7/3 MeOH/H_2O (v/v); flow rate 1 ml min^{-1}; retention time (min); 7.7], and negative FAB-MS m/z: 2003 $[M-H]^-$, 1401 $[M-H-602]^-$.

Alkaline Hydrolysis of 5. A solution of **5** (30 mg) in MeOH (1 ml) and 3% KOH (1 ml) in MeOH was refluxed for 10 h. The reaction mixture was diluted with H_2O (10 ml), and aqueous solution was fractionated by chromatography on a column of MCI gel CHP-20P (0→100% MeOH) to give three fractions (frs. 1—3). Fractions 2 and 3 were further purified by silica-gel column to give compound **7** (5.3 mg) and compound **6** (7.5 mg), respectively.

Compound 6: A white amorphous powder, $[\alpha]_D$ −11.9° (c=0.28, MeOH). HR negative FAB-MS m/z 1089.5486 $[M-H]^-$ ($C_{53}H_{85}O_{23}$, Calcd for M, 1089.5482). ^1H NMR (pyridine-d_5) δ=0.92, 1.01, 1.12, 1.17, 1.19, 1.40, 1.73 [tertiary methyl groups]; 5.30 (1H, br s, H-12), 5.38 (1H, d, J=8.1 Hz), 5.09 (1H, d, J=7.7 Hz), 4.87 (1H, d, J=7.6 Hz), 4.84 (1H, d, J=7.7 Hz) [anomeric protons]; 1.51 (3H, d, J=6.2 Hz) [secondary methyl proton]. ^{13}C NMR (pyridine-d_5): Tables 1 and 2.

Compound 7: A white amorphous powder, $[\alpha]_D$ −16.8° (c=0.32, MeOH). HR positive FAB-MS m/z 353.1577 $[M+Na]^+$ ($C_{16}H_{26}O_7Na$, Calcd for M, 353.1577). ^1H NMR (pyridine-d_5) δ=7.18 (1H, m, H-3), 2.48 (1H, m, H-4), 1.81 (1H, m, H-5), 6.24 (1H, dd, J=11.0, 17.6 Hz, H-7), 5.44 (1H, d, J=17.6 Hz, H-8), 5.23 (1H, d, J=11.0 Hz, H-8), 2.01 (3H, s, H-9), 1.56 (3H, s, H-10), 4.99 (1H, d, J=7.7 Hz, qui H-1), 1.51 (3H, d, J=6.2 Hz, qui H-6). ^{13}C NMR (pyridine-d_5): Table 4.

Acidification of 6. A solution of **6** (1 mg) in H_2O (1 ml) was passed through a column of Amberlite IR-120B and washed with H_2O and MeOH. The filtrate and washing were combined, and the mixture was evaporated to dryness. The residue was identified by TLC to be Julibroside A_1 (**1a**)[1] [R_f 0.30 ($CHCl_3$–MeOH–H_2O=7:3:0.5), 0.19 (BuOH–AcOH–H_2O=4:1:5, upper layer)].

Enzymatic Hydrolysis of 7. Compoud **7** (5 mg) and β-glucosidase (100 units) were dissolved in acetate buffer (100 mM, pH 5.0, 1.0 ml) and incubated at 37 °C for 2 d. The reaction mixture was separated by MCI gel CHP-20P (0→100% MeOH) to give compound **8** (3.2 mg). A pale yellow oil, $[\alpha]_D$ +14.8° (c=0.24, MeOH). ^1H NMR ($CDCl_3$) δ=6.88 (1H, m, H-3), 2.20 (1H, m, H-4), 1.66 (1H, m, H-5), 5.91 (1H, dd, J=10.6, 17.2 Hz, H-7), 5.24 (1H, d, J=17.2 Hz, H-8), 5.10 (1H, d, J=10.6 Hz, H-8), 1.82 (3H, s, H-9), 1.32 (3H, s, H-10). ^{13}C NMR ($CDCl_3$): Table 4.

Julibroside II (2). A white amorphous powder, $[\alpha]_D$ −27.7° (c=0.49, MeOH). Positive FAB-MS (uncorrected) m/z 2178 $[M+Na]^+$, negative FAB-MS (uncorrected) m/z 2154 $[M-H]^-$, 1552 $[M-H-602]^-$. HR positive FAB-MS m/z 2179.0342 $[M+Na]^+$ ($^{12}C_{101}{}^{13}CH_{162}O_{48}Na$, Calcd for M, 2179.0167). ^1H NMR (pyridine-d_5) δ=1.00 (2×CH_3),

1.01, 1.07, 1.14, 1.18, 1.34, 1.57, 1.81, 1.90 ($2\times CH_3$) [tertiary methyl groups]; 7.05 (1H, m), 6.39 (1H, m), 6.25—6.33 (2H, m), 5.56 (1H, br s), 5.54 (1H, d, J=17.6 Hz), 5.46 (1H, d, J=17.6 Hz), 5.32 (1H, d, J=10.4 Hz), 5.26 (1H, d, J=10.4 Hz) [olefinic protons]; 6.25 (1H, d, J=7.8 Hz), 6.01 (1H, d, J=8.1 Hz), 5.94 (1H, br s), 5.39 (1H, d, J=7.6 Hz), 5.29 (1H, d, J=8.4 Hz), 5.04 (1H, d, J=6.9 Hz), 4.99 (1H, d, J=8.1 Hz), 4.90 (1H, d, J=7.7 Hz), 4.85 (1H, d, J=7.7 Hz) [anomeric protons]; 1.35 (3H, d, J=6.2 Hz), 1.51 (3H, d, J=6.2 Hz), 1.58 (3H, d, J=6.2 Hz), 1.81 (3H, br s) [secondary methyl protons]. ^{13}C NMR (pyridine-d_5): Tables 1, 2, 3, and 4.

Alkaline Hydrolysis of 2. A solution of **2** (1 mg) in 3% KOH (0.5 ml) in MeOH was refluxed for 10 h. The reaction mixture was evaporated to dryness and diluted with H_2O; an aqueous solution was then passed through a column of Amberlite® IR-120B and washed with H_2O and MeOH. The filtrate and washing were combined, and the mixture was evaporated to dryness. The residue was purified by MCI gel to obtain two compounds as major products. These compounds were identified by TLC with Julibroside A_2 (**2a**)[1] [R_f 0.56 ($CHCl_3$–MeOH–H_2O= 7:3:0.5), 0.34 (BuOH–AcOH–H_2O=4:1:5, upper layer)] and **7** [R_f 0.62 ($CHCl_3$–MeOH–H_2O = 7:3:0.5), 0.78 (BuOH–AcOH–H_2O=4:1:5, upper layer)], respectively.

Julibroside III (3). A white amorphous powder, $[\alpha_D]$ −16.4° (c=0.22, MeOH). Positive FAB-MS (uncorrected) m/z 2219 $[M+Na]^+$, negative FAB-MS (uncorrected) m/z 2196 $[M-H]^-$, 1593 $[M-H-602]^-$. HR negative FAB-MS m/z 2196.0466 $[M-H]^-$ ($^{12}C_{103}{}^{13}CH_{164}NO_{48}$, Calcd for M, 2196.0456). ^{1}H NMR (pyridine-d_5) δ=0.95, 1.00, 1.04, 1.07, 1.18, 1.19, 1.51, 1.55, 1.84, 1.88, 1.90 [tertiary methyl groups]; 2.12 ($\underline{CH_3}$–NHCOO–), 7.04 (1H, m), 6.90 (1H, m), 6.21—6.36 (2H, m), 5.61 (1H, br s), 5.46 (1H, d, J=17.5 Hz), 5.43 (1H, d, J=17.6 Hz), 5.39 (1H, d, J=11.7 Hz), 5.27 (1H, d, J=11.7 Hz) [olefinic protons]; 6.19 (1H, d, J=6.2 Hz), 6.06 (1H, d, J=8.1 Hz), 5.92 (1H, br s), 5.35 (1H, d, J=7.7 Hz), 5.25 (1H, d, J=7.3 Hz), 5.06 (1H, d, J=8.0 Hz), 5.04 (1H, d, J=8.1 Hz), 4.99 (1H, d, J=7.3 Hz), 4.88 (1H, d, J=7.7 Hz) [anomeric protons], 1.35 (3H, d, J=5.9 Hz), 1.49 (3H, d, J=6.6 Hz), 1.59 (3H, d, J=5.1 Hz), 1.78 (3H, d, J=5.9 Hz) [secondary methyl protons]; 8.88 (1H, d, J=8.8 Hz, NH). ^{13}C NMR (pyridine-d_5): Tables 1, 2, 3, and 4.

Alkaline Hydrolysis of 3. A sample of **3** (1 mg) was saponified as described above to give two compounds. These compounds were identified by TLC to be Julibroside A_3 (**3a**)[1] [R_f 0.49 ($CHCl_3$–MeOH–H_2O= 7:3:0.5), 0.28 (BuOH–AcOH–H_2O=4:1:5, upper layer)] and **7** [R_f 0.62 ($CHCl_3$–MeOH–H_2O = 7:3:0.5), 0.78 (BuOH–AcOH–H_2O=4:1:5, upper layer)], respectively.

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