Cytotoxic Glycosides from *Albizia julibrissin*

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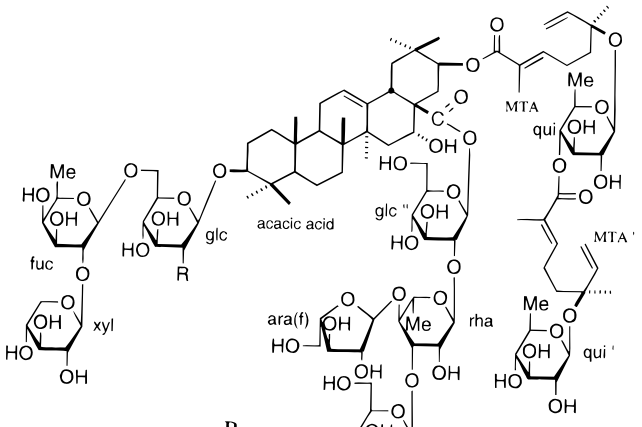
During the course of a study of leguminous plants, cytotoxicity was demonstrated by the crude saponin fraction of *Albizia julibrissin*. Following chromatographic purification, the structures of three novel saponins, julibrosides I−III (**1**−**3**), inclusive of a cytotoxic principle, were elucidated. A comparison of the cytotoxicity of julibrosides (**1**−**3**) and their prosapogenins (**4**−**15**) prepared by alkaline hydrolysis clearly indicated that both an α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester unit and a monoterpene−quinovopyranosyl moiety are crucial substituents for cytotoxicity among this class of compounds. The hydroxy group at C-16 of aglycon may play an important role in mediating cytotoxicity, and the *N*-acetyl-glucosamine moiety at C-3 seems to enhance activity because **3** showed the strongest cytotoxicity.

The dried stem bark of *Albizia julibrissin* Durazz. (Leguminosae) (Albiziae Cortex) is used as a tonic in the People's Republic of China and Japan. During our study on the constituents of leguminous plants, we have reported the isolation of six new triterpenoidal saponins, julibrosides A_{1-4}, B_1, and C_1,[1] and three novel acylated triterpenoidal saponins, julibrosides I−III (**1**−**3**),[2] as well as a prosapogenin, prosapogenin 8 (**11**),[2] from *A. julibrissin*. Because a crude saponin fraction obtained from *A. julibrissin* showed significant (IC_{50} 12.8 μg/mL) cytotoxicity against the KB cell line, we have determined the cytotoxicity profile of the purified saponins. Furthermore, in order to investigate the relationship between cytotoxicity and compound structure, we have prepared several prosapogenins by alkaline hydrolysis and also tested them for cytotoxicity.



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Julibroside I (**1**) -O-glc'
Julibroside II (**2**) -OH
Julibroside III (**3**) -NHAc

Results and Discussion

The fresh stem bark of *Albizia julibrissin* was extracted with MeOH, and the extract was partitioned with *n*-BuOH and H_2O. The aqueous extract was subjected to MCI gel CHP-20P column chromatography to give a crude saponin fraction. In order to further study this class of triterpenoid glycoside, the crude saponin fraction was saponified first using 3% KOH in MeOH. The saponified prosapogenins were separated by column chromatography using MCI gel CHP-20P, Bondapak C_{18}, and Si gel to afford compounds designated as prosapogenins 1−7 (**4**−**10**). Prosapogenins 5 (**8**), 6 (**9**), and 7 (**10**) were identified as julibrosides A_1, A_2, and A_3, respectively, by comparing with each originally isolated sample.[1]

Prosapogenin 1 (**4**) exhibited a $[M + Na]^+$ ion at *m/z* 951 in the positive-ion FABMS. On comparative analysis of the ^{13}C-NMR signals of **4** and **9** (Table 1), they were in good agreement, except for the aglycon moiety, which was identified as acacic acid in the case of **4**.[1] Therefore, the structure of prosapogenin 1 (**4**) was determined to be 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosylacacic acid.

Prosapogenin 2 (**5**) exhibited a $[M + Na]^+$ ion at *m/z* 978 and a $[M + H]^+$ ion at *m/z* 956 in the positive-ion FABMS. Upon acid hydrolysis with 2 N HCl, **5** gave an acacic acid lactone unit, which was identified with an authentic sample,[1] as well as xylose, arabinose, and *N*-acetyl-glucosamine as component sugars. When the ^{1}H- and ^{13}C-NMR signals of **5** were compared with those of the aglycon portion of **4** (Table 1), they were in good agreement, whereas the sugar signals of **5** were similar to those of **10**, except for the appearance of arabinopyranosyl signals instead of fucopyranosyl signals. Therefore, the structure of **5** was established as 3-*O*-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-β-D-2-acetoamido-2-deoxy-glucopyranosylacacic acid.

Prosapogenin 3 (**6**) exhibited a $[M + Na]^+$ ion at *m/z* 919 in the positive-ion FABMS, suggesting it was the desmethyl derivative of **9**. Furthermore, fragment ion peaks at *m/z* 633 $[M + H - xyl - ara]^+$ and *m/z* 471 $[M + H - xyl - ara - glc]^+$ were observed. When the ^{13}C-NMR signals of **6** (Table 1) were compared with those of **9**, the aglycon signals were found to be superimposable. The sugar signals were identical to those of julibroside J_1, which was recently obtained from *A. julibrissin* by Ma *et al.*[3] Therefore, the structure of **6**

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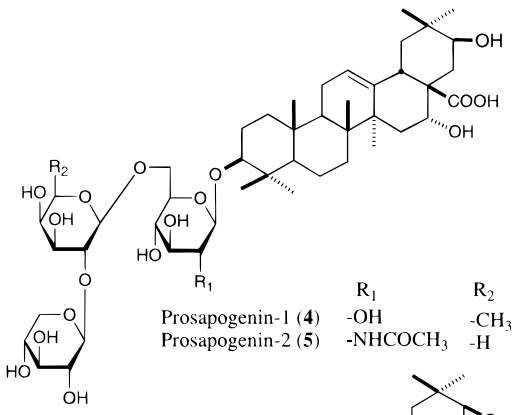
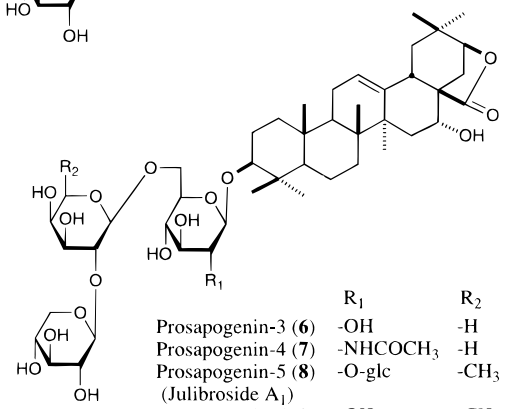


	R_1	R_2
Prosapogenin-1 (**4**)	-OH	$-CH_3$
Prosapogenin-2 (**5**)	$-NHCOCH_3$	-H



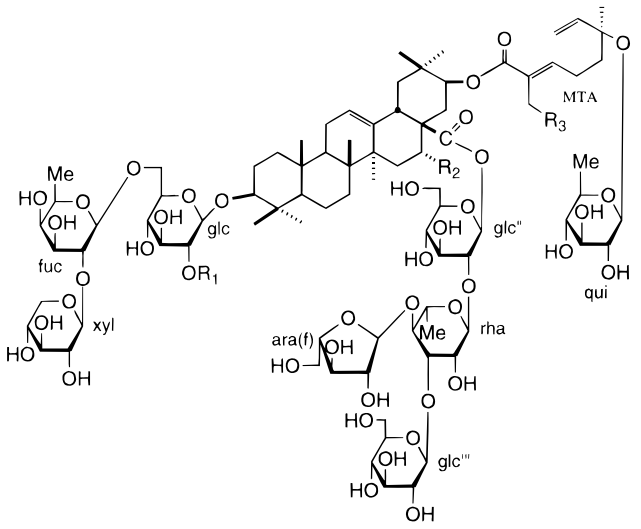
	R_1	R_2
Prosapogenin-3 (**6**)	-OH	-H
Prosapogenin-4 (**7**)	$-NHCOCH_3$	-H
Prosapogenin-5 (**8**) (Julibroside A_1)	-O-glc	$-CH_3$
Prosapogenin-6 (**9**) (Julibroside A_2)	-OH	$-CH_3$
Prosapogenin-7 (**10**) (Julibroside A_3)	$-NHCOCH_3$	$-CH_3$

was determined to be 3-*O*-*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranosylacacic acid lactone.

Prosapogenin 4 (**7**) exhibited a $[M + Na]^+$ ion at *m/z* 960 and a $[M + H]^+$ ion at *m/z* 938 in the positive-ion FABMS. Furthermore, fragment ion peaks at *m/z* 806 $[M + H - xyl]^+$, 674 $[M + H - xyl - ara]^+$, and *m/z* 468 $[xyl + ara + glcNAc + H]^+$ were observed. When the ^{13}C-NMR signals of **7** (Table 1) were compared with those of **5** and **6**, resonances due to the aglycon moieties of **6** and **7** were superimposable, whereas the sugar moiety linked at C-3 was found to be identical with that of **5**. Therefore, the structure of **7** was determined to be 3-*O*-*β*-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-*β*-D-2-acetoamido-2-deoxy-glucopyranosylacacic acid lactone.

After alkaline hydrolysis of the crude saponin fraction with saturated $NaHCO_3$ in MeOH, the hydrolysate was separated by successive column chromatography over MCI gel CHP-20P, Bondapak C_{18}, Chromatorex ODS-DU 3050MT, and Si gel to provide prosapogenins 8−12 (**11**−**15**), among which the structure of prosapogenin 8 (**11**) has been reported in an ealier paper.[2]

Prosapogenin 9 (**12**) exhibited a $[M - H]^-$ ion at *m/z* 1841 in the negative-ion FABMS, and significant fragment peaks at *m/z* 1709 $[M - H - ara]^-$, 1679 $[M - H - glc]^-$, and 1239 $[M - H - 602]^-$ were observed. By comparing the FABMS of **12** and **2**, the molecular ion peak of **12** was 312 mass units less than that of **2**. This indicated that **12** lacked the quinovosyl monoterpene unit found in **2**. The similar fragment ion peak[2] at $[M - H - 602]^-$ suggested that the sugar moiety of C-28 was the same as that of **2**. When the ^{1}H- and ^{13}C-NMR signals of **12** were compared with those of **2** (Table 2), they were in good agreement, except for lack of a



	R_1	R_2	R_3
Prosapogenin-8 (**11**)	-glc'	-OH	-H
Prosapogenin-9 (**12**)	-H	-OH	-H
Prosapogenin-10 (**13**)	-H	-OH	-OH
Prosapogenin-11 (**14**)	-H	-H	-H
Prosapogenin-12 (**15**)	-glc'	-H	-H

terminal quinovopyranosyl monoterpene unit affixed to C-21. Inasmuch as signals for the remaining units were in accord with those of **11**, the structure of **12** was determined as 21-*O*-[(*6S*)-2-*trans*-2,6-dimethyl-6-*O*-*β*-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

Prosapogenin 10 (**13**) exhibited a $[M - H]^-$ ion at *m/z* 1857 in the negative-ion FABMS, along with major fragment ion peaks at *m/z* 1695 $[M - H - glc]^-$ and *m/z* 1255 $[M - H - 602]^-$. By comparison of the FABMS data of **13** and **12**, the molecular ion peak of **13** was seen to be 16 mass units larger than that of **12**. When the ^{1}H- and ^{13}C-NMR signals of **13** were compared with those of **12** (Table 2), they were in good agreement, except for the tertiary methyl group of a monoterpene acid portion of the molecule. Thus, in the ^{13}C-NMR spectrum of **13**, a hydroxymethyl carbon (*δ* 56.1) was apparent, while a tertiary methyl carbon (*δ* 12.5) was no longer present. Therefore, the structure of **13** was determined as 21-*O*-[(*6S*)-2-*trans*-2-methyl-6-hydroxymethyl-6-*O*-*β*-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

Prosapogenin 11 (**14**) exhibited a $[M - H]^-$ ion at *m/z* 1825 in the negative-ion FABMS, suggesting it to be the dehydroxy compound of **12**. Furthermore, the fragment ion peaks at *m/z* 1663 $[M - H - glc]^-$ and 1223 $[M - H - 602]^-$ were 16 mass units less than analogous fragment peaks for **12**. When the ^{1}H- and ^{13}C-NMR signals of **14** were compared with those of **12** (Table 2), they were in good agreement, except for those of the aglycon moieties. From the ^{13}C-NMR spectrum, the aglycon moiety of **14** was identified to be machaerinic acid, which is a deoxy derivative of acacic acid.[1] Therefore, the structure of **14** was determined as 21-*O*-[(*6S*)-

Table 1. ^{13}C-NMR Chemical Shifts for Compounds **4**−**7**[a]

carbon	4	5	6	7	carbon	4	5	6	7
aglycon					sugar				
1	38.7	38.3	38.6	38.6	glc 1	106.6		105.3	
2	26.7	26.2	26.4	26.4	2	75.7		74.9	
3	88.2	88.9	89.3	89.0	3	78.2		76.9	
4	39.5	38.9	39.3	39.2	4	71.2		71.0	
5	55.8	55.5	55.9	55.9	5	76.6		75.7	
6	18.4	18.2	18.2	18.4	6	69.9		68.9	
7	33.4	33.2	32.3	32.5					
8	39.8	39.5	40.3	40.3	glcNAc 1		104.7		104.6
9	47.1	46.8	47.1	47.2	2		57.3		57.6
10	36.6	36.6	36.8	36.9	3		74.9		75.2
11	23.8	23.5	23.6	23.7	4		72.3		72.4
12	122.6	122.7	124.8	124.7	5		75.7		76.1
13	144.3	144.4	139.8	140.0	6		69.9		69.3
14	41.9	41.7	43.3	43.3	NHCO*CH*$_3$		23.2		23.5
15	35.7	35.3	37.5	38.0	NH*CO*CH$_3$		171.0		171.3
16	74.2	73.8	67.1	66.8					
17	51.6	51.4	50.0	50.0	fuc 1	103.2			
18	40.8	40.6	41.7	41.7	2	82.0			
19	48.3	48.1	43.3	43.0	3	75.1			
20	37.0	36.7	33.9	34.1	4	72.1			
21	73.4	73.5	84.3	83.6	5	71.5			
22	41.7	41.2	26.9	27.1	6	17.4			
23	28.1	27.8	28.6	28.6					
24	15.5	15.2	15.6	15.6	ara 1		101.7	102.2	102.3
25	17.0	16.7	16.1	16.2	2		80.0	79.9	80.3
26	17.1	17.1	16.9	17.0	3		67.0	67.3	67.0
27	27.2	26.9	28.1	28.0	4		72.0	71.9	72.3
28	179.2	179.3	182.5	181.6	5		63.8	64.2	63.8
29	29.9	29.7	28.9	28.9					
30	18.3	18.0	23.4	24.1	xyl 1	106.8	105.7	106.0	106.0
					2	75.7	75.2	74.9	75.4
					3	77.4	77.3	77.2	77.5
					4	70.6	70.4	70.2	70.7
					5	67.0	66.8	66.5	67.0

[a] Values are recorded at 100 MHz in pyridine-d_5.

2-*trans*-2,6-dimethyl-6-*O*-*β*-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosylmachaerinic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

Prosapogenin 12 (**15**) exhibited a $[M - H]^-$ ion at *m/z* 1987 in the negative-ion FABMS, thereby indicating that an additional hexose unit was present in this molecule when compared to **14**. Furthermore, fragment ion peaks at *m/z* 1852 $[M - H - glc]^-$, 1385 $[M - H - 602]^-$, and 1223 $[M - H - 602 - glc]^-$ were, respectively, 162 mass units larger than those of **14**. When the ^{1}H- and ^{13}C-NMR signals of **15** were compared with those of **14** (Table 2), they were in good agreement, except for the sugar moiety at C-3, although the sugar unit linked at C-3 was the same as that of **1**. Therefore, the structure of **15** was determined 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-glucopyranosylmachaerinic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

The cytotoxicity of julibrosides I−III (**1**−**3**) and their prosapogenins 1−12 (**4**−**15**) was examined against the KB cell line, with the IC_{50} values obtained listed in Table 3. It is apparent that the prosapogenins obtained by alkaline (KOH) hydrolysis (**4**−**10**) are completely inactive. On the other hand, some of the acylated triterpene glycosides (**3**, **11**−**13**) showed significant cytotoxicity. In the case of the genuine saponins (**1**−**3**), only julibroside III (**3**) with a *N*-acetyl-glucosamine unit showed activity. For the prosapogenins obtained by mild alkaline ($NaHCO_3$) hydrolysis (**11**−**15**), the compounds possessing trisaccharide units at C-3 (**12**−**14**) were more active than those with tetrasaccharide moieties (**11** and **15**). The prosapogenins having an acacic acid-type aglycon (**11**−**13**) were much more active than those based on machaerinic acid (**14** and **15**).

A comparison of the cytotoxicity of the julibrosides (**1**−**3**) and their prosapogenins (**4**−**15**) (Table 3, Figure 1) clearly indicated that both the α-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester and monoterpene−quinovopyranosyl moieties are crucial substituents required for cytotoxicity. However, the terminal monoterpene−quinovopyranosyl moiety seems not to be necessary for cytotoxicity against KB cells owing to the inactivity of **1** and **2**. The hydroxy group at C-16 of the aglycon may play an important role in mediating cytotoxicity among these compounds. Furthermore, the *N*-acetyl-glucosamine moiety at C-3 seems to enhance activity, because **3** showed the strongest cytotoxicity.

Experimental Section

General Experimental Procedures. The optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded with a JEOL JIR-6500W FT-IR spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 and/or an α-500 FT-NMR spectrometer, and chemical shifts are given on a *δ* (ppm) scale with Me_4Si as the internal standard. The FABMS were measured with a JEOL DX-300 and/or a JEOL SX102A spectrometer. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). GC was performed using a Hewlett

Table 2. ^{13}C-NMR Chemical Shifts for Compounds **11**−**15**[a]

carbon	11	12	13	14	15	carbon	11	12	13	14	15
aglycon						sugar (C-28)					
C-1	38.7	38.7	38.7	38.8	38.7	glc″ 1	95.5	95.4	95.5	95.4	95.3
2	26.6	26.6	26.6	26.7	26.7	2	78.9	79.8	78.9	79.0	78.9
3	88.5	88.2	88.2	87.9	88.1	3	76.8	76.9	76.9	76.7	76.9
4	39.5	39.4	39.4	39.6	39.6	4	72.0	72.0	71.6	71.7	72.0
5	55.9	55.9	55.8	55.8	55.8	5	78.2	78.0	78.2	78.3	78.2
6	18.5	18.5	18.5	18.7	18.6	6	62.6	62.3	62.4	62.3	62.6
7	33.4	33.4	33.4	33.4	33.3	rha (1 → 2) glc″					
8	39.9	39.9	40.0	39.9	39.8	1	101.7	101.6	101.6	101.6	101.6
9	46.9	47.0	47.0	48.0	47.9	2	70.3	70.4	70.3	70.6	70.6
10	36.9	36.9	36.9	37.0	36.9	3	78.8	78.7	78.7	78.9	78.2
11	23.7	23.7	23.8	23.9	23.8	4	84.2	84.2	84.2	84.6	84.5
12	122.9	122.8	122.9	122.7	122.9	5	69.0	68.9	69.0	68.8	68.8
13	143.2	143.2	143.2	142.9	142.8	6	18.7	18.7	18.7	18.4	18.4
14	41.8	41.8	41.8	42.2	42.1	glc‴ (1 → 3) rha					
15	35.7	35.7	35.7	28.7	28.4	1	105.5	105.5	105.5	105.6	105.5
16	73.7	73.6	73.7	24.7	24.6	2	75.0	75.0	75.0	75.1	75.0
17	51.5	51.4	51.5	48.6	48.5	3	77.9	77.9	78.0	78.3	78.0
18	40.8	40.8	40.8	41.2	41.1	4	71.1	71.1	71.1	71.2	71.6
19	47.7	47.6	47.7	46.4	46.3	5	78.2	78.0	78.2	78.2	78.0
20	35.1	35.1	35.3	35.4	35.3	6	62.6	62.5	62.6	62.6	71.6
21	76.7	76.5	76.5	76.5	76.5	ara (1 → 4) rha					
22	36.2	36.2	36.2	36.3	36.2	1	110.8	110.8	110.8	110.8	110.7
23	27.9	28.0	28.0	28.0	27.8	2	81.8	81.9	81.9	81.9	81.9
24	15.7	15.7	15.7	15.7	15.7	3	78.2	78.0	78.2	78.3	78.2
25	16.7	17.0	17.0	17.1	16.8	4	85.2	85.2	85.2	84.7	84.6
26	17.0	17.2	17.2	17.3	17.0	5	61.8	61.8	61.8	61.9	61.9
27	27.1	27.1	27.1	25.8	25.7						
28	174.3	174.3	174.3	174.8	174.7	MTA[b] 1	167.6	167.6	167.4	167.5	167.4
29	29.0	28.9	29.0	28.7	28.6	2	128.2	128.2	133.5	127.9	127.9
30	19.0	19.0	19.0	18.8	18.7	3	142.3	142.3	145.3	143.0	142.9
sugar (C-3)						4	23.45	23.40	23.40	23.60	23.51
glc 1	104.7	106.5	106.5	106.5	104.7	5	40.5	40.3	40.7	40.3	40.3
2	82.6	75.5	75.5	75.6	82.9	6	79.3	79.3	79.4	79.4	79.3
3	76.8	78.0	78.2	78.3	76.7	7	143.9	143.9	143.8	144.0	143.9
4	71.5	71.5	71.4	71.7	71.6	8	114.7	114.7	114.7	114.8	114.7
5	77.4	77.3	77.3	77.4	77.4	9	12.5	12.5	56.1	12.5	12.4
6	69.6	69.8	69.8	69.8	69.6	10	23.54	23.50	26.60	23.60	23.54
glc′ (1 → 2) glc											
1	105.5				105.6	qui 1	99.1	99.1	99.1	99.2	99.1
2	75.7				75.3	2	75.3	75.3	75.3	75.4	75.3
3	78.0				78.0	3	78.2	78.0	78.2	78.3	78.2
4	71.1				71.2	4	76.7	76.6	76.7	76.7	76.7
5	78.0				78.0	5	72.4	72.4	72.4	72.5	72.4
6	62.3				62.2	6	18.7	18.7	18.7	18.7	18.7
fuc (1 → 6) glc											
1	103.2	103.1	103.1	103.3	103.3						
2	82.0	81.9	81.9	82.2	82.3						
3	75.2	75.2	75.2	75.3	75.2						
4	72.4	72.4	72.0	72.1	72.4						
5	71.0	71.0	71.0	71.2	71.1						
6	17.2	17.0	17.0	17.1	17.2						
xyl (1 → 2) fuc											
1	106.8	106.6	106.7	106.9	106.9						
2	76.3	75.6	75.6	75.8	75.8						
3	77.8	77.8	77.8	78.0	77.8						
4	70.6	70.5	70.6	70.6	70.6						
5	67.0	67.0	67.0	67.1	67.0						

[a] Values are recorded 100 MHz in pyridine-d_5. [b] MTA = Monoterpenoid acid moiety.

Packard HP5890A. The GC conditions were as follows: column, OV-1 (0.5-μm film bonded, 0.32 × 30 m); column oven temperature, 230 °C; injection port temperature, 270 °C; detection port temperature, 270 °C; carrier gas, He (2.2 kg/cm^2). Column chromatography was carried out on Kieselgel 60 (70−230 mesh and 230−400 mesh), MCI gel CHP-20P (Mitsubishi Chemical Industries, Tokyo, Japan), Bondapak C_{18} (Waters), Wako gel LP60 C_{18} (Wako Pure Chemical Industries, Osaka, Japan), Sephadex LH-20 (Pharmacia), and Chromatorex ODS-DU 3050MT (Fuji Silysia, Kasugai, Japan).

Plant Material. The fresh stem bark of *A. julibrissin* Durazz. was collected at the Botanical Garden of Kumamoto University. Albiziae Cortex, which is the commercially available stem bark of *A. julibrissin*, was purchased from Uchida Wakanyaku Co., Ltd. (Tokyo, Japan).

Cytotoxicity Bioassays Against KB Cells. The tetrazolium-based semiautomated colorimetric assay (MTT assay) developed by Carmichael *et al.*[4] was used for the in vitro assay of cytotoxicity to KB cells.

Extraction and Isolation. Commercially available Albiziae Cortex (10 kg) was extracted with MeOH twice under reflux. The combined extract was concentrated (537 g) and partitioned with EtOAc and 40% MeOH (380 g). A part of the 40% MeOH extract (180 g) was subjected to column chromatography over MCI gel CHP-

Table 3. Cytotoxicity of Saponins and Prosapogenins from *Albizia julibrissin*

compound	KB cell cytotoxicity [IC_{50}, μg/mL (μM)]
julibroside I (**1**)	>10
julibroside II (**2**)	>10
julibroside III (**3**)	1.9 (0.9)
prosapogenin 1 (**4**)	>10
prosapogenin 2 (**5**)	>10
prosapogenin 3 (**6**)	>10
prosapogenin 4 (**7**)	>10
prosapogenin 5 (**8**)	>10
prosapogenin 6 (**9**)	>10
prosapogenin 7 (**10**)	>10
prosapogenin 8 (**11**)	9.1 (4.5)
prosapogenin 9 (**12**)	2.9 (1.6)
prosapogenin 10 (**13**)	3.7 (2.0)
prosapogenin 11 (**14**)	3−10 (1.6−5.5)
prosapogenin 12 (**15**)	>10

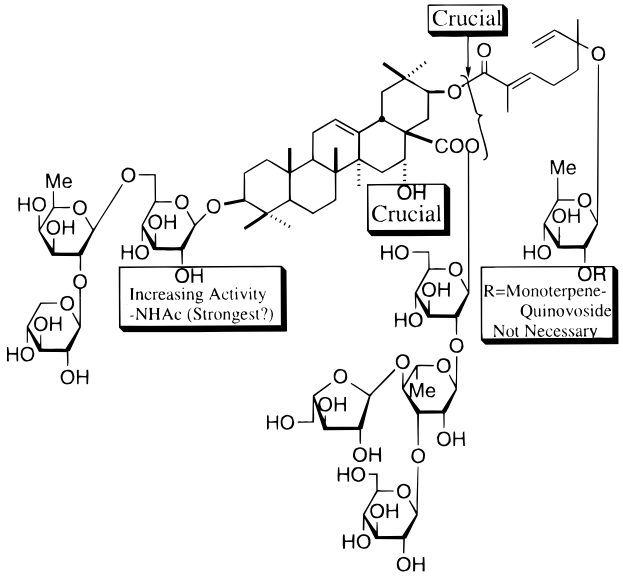


Figure 1. Structure−activity relationships for KB cell cytotoxicity of saponins and prosapogenins.

20P using H_2O→100% MeOH to give fractions 1 to 4. Fraction 3 (32.5 g) was dissolved in 3% KOH−MeOH (50 mL) and refluxed for 10 h. The reaction mixture was carefully neutralized with 1 N HCl and further separated by chromatography over MCI gel CHP-20P (H_2O→100% MeOH), Bondapak C_{18} (H_2O→MeOH), and Si gel [$CHCl_3$−MeOH−H_2O (7:3:0.5)] to afford prosapogenins 3 (**6**, 10 mg), 4 (**7**, 11 mg), 5 (**8**, 5 mg), 6 (**9**, 24 mg), and 7 (**10**, 18 mg). Fraction 2 (22.3 g) was further separated by MCI gel CHP-20P (H_2O→MeOH) and Bondapak C_{18} (H_2O→MeOH) to afford an acylated saponin fraction (490 mg). The native glycosides were saponified in the same manner as described above to provide prosapogenins 1 (**4**, 10 mg) and 2 (**5**, 9 mg).

Prosapogenin 1 (4): a white amorphous powder; $[\alpha]_D$ −8.8° (*c* 0.50, MeOH); IR ν_{max} (KBr) cm^{-1} 3465 (OH), 1645 (COOH) cm^{-1}; ^{1}H NMR (in pyridine-d_5) δ 0.90, 1.03 × 2, 1.35 × 2, 1.44, 1.94 (each 3H, s, *tert*-methyl), 1.51 (3H, d, J = 6.6 Hz, fuc − 6), 4.95 (1H, d, J = 7.0 Hz, xyl − 1), 5.03 (1H, d, J = 8.4 Hz, glc − 1), 5.09 (1H, d, J = 6.6 Hz, fuc − 1), 5.65 (1H, br s, H-12); ^{13}C-NMR (pyridine-d_5) data, see Table 1; positive-ion FABMS m/z $[M + Na]^+$ 951.

Prosapogenin 2 (5): a white amorphous powder; $[\alpha]_D$ +1.0° (*c* 0.50, MeOH); IR ν_{max} (KBr) cm^{-1} 3470 (OH), 1645 (COOH) cm^{-1}; ^{1}H NMR (in pyridine-d_5) δ 0.86, 0.99, 1.01, 1.20, 1.37, 1.45, 1.91 (each 3H, s, *tert*-methyl), 2.16 (3H, s, NHCOCH_3), 3.41 (1H, dd, J = 4.6, 11.5 Hz, H-3), 5.00 (1H, d, J = 7.0 Hz, xyl − 1), 5.05 (1H, d, J = 8.4 Hz, glcNAc − 1), 5.17 (1H, d, J = 5.1 Hz, ara − 1), 5.64 (1H, br s, H-12), 8.95 (1H, d, J = 8.8 Hz, NHCOCH$_3$); ^{13}C-NMR (pyridine-d_5) data, see Table 1; positive-ion FABMS m/z $[M + Na]^+$ 978, $[M + H]^+$ 956, $[M + H - xyl]^+$ 824.

Acid Hydrolysis of 5. A small amount of **5** was hydrolyzed by 2 N HCl in a heating bath. After filtration of the mixture, the precipitate was identified as acacic acid lactone by Si gel TLC [$CHCl_3$−MeOH (10:1), hexane−acetone (1:1)],[1] while the filtrate was treated with a N_2 stream to give a residue. The residue was converted to the Me_3Si ether of methyl 2-(polyhydroxyalkyl)-thiazolidine-4-(*R*)-carboxylate and checked by GC,[5] which showed the sugars to be D-xylose, L-arabinose, and D-*N*-acetyl-glucosamine.

Prosapogenin 3 (6): a white amorphous powder; $[\alpha]_D$ −15.5° (*c* 0.49, MeOH); IR ν_{max} (KBr) cm^{-1} 3415 (OH), 1765 (γ-lactone) cm^{-1}; ^{1}H-NMR (in pyridine-d_5) δ 0.80, 0.83, 1.02, 1.05, 1.09, 1.30, 1.38 (each 3H, s, *tert*-methyl), 3.49 (1H, *m*, H-3), 4.90 (1H, d, J = 8.1 Hz, glc − 1), 4.95 (1H, d, J = 7.1 Hz, xyl − 1), 5.05 (1H, d, J = 5.1 Hz, ara − 1), 5.36 (1H, br s, H-12); ^{13}C-NMR (pyridine-d_5) data, see Table 1; positive-ion FABMS m/z $[M + Na]^+$ 919, $[M + H - xyl- ara]^+$ 633, $[M + H - xyl - ara - glc]^+$ 471.

Prosapogenin 4 (7): a white amorphous powder; $[\alpha]_D$ −10.1° (*c* 0.47, MeOH); IR ν_{max} (KBr) cm^{-1} 3455 (OH), 1755 (γ-lactone) cm^{-1}; ^{1}H-NMR (in pyridine-d_5) δ 0.79 × 2, 0.96, 0.99, 1.08, 1.22, 1.39 (each 3H, s, *tert*-methyl), 2.24 (3H, s, NHCOCH_3), 3.41 (1H, *m*, H-3), 4.95 (1H, d, J = 7.3 Hz, xyl − 1), 5.06 (1H, d, J = 8.4 Hz, glcNAc − 1), 5.14 (1H, d, J = 5.1 Hz, ara − 1), 5.33 (1H, br s, H-12), 9.18 (1H, d, J = 8.8 Hz, NHCOCH$_3$); ^{13}C-NMR (pyridine-d_5) data, see Table 1; positive-ion FABMS m/z $[M + Na]^+$ 960, $[M + H]^+$ 938, $[M + H - xyl]^+$ 806, $[M + H - xyl - ara]^+$ 674, $[xyl + ara + glcNAc + H]^+$ 468.

The fresh stem bark of *A. julibrissin* (6.3 kg) was extracted with MeOH twice under reflux. The combined extract (379 g) was concentrated and partitioned with *n*-BuOH and H_2O. The aqueous extract (294 g) was subjected to MCI gel CHP-20P column chromatography using H_2O→MeOH to give a crude saponin mixture (52 g). A portion (4 g) in saturated $NaHCO_3$ in MeOH (400 mL) was refluxed for 1 h. The reaction mixture was evaporated to dryness and separated, in turn, by MCI gel CHP-20P (H_2O→MeOH), Bondapak C_{18} (20%MeOH→70%MeOH), Chromatorex ODS (60%MeOH→70%MeOH), and Si gel column chromatography [$CHCl_3$−MeOH−H_2O (7:3:0.5→6:4:1)] to afford prosapogenin 8 (**11**, 104 mg) as the major component,[2] along with prosapogenin 9 (**12**, 34 mg), prosapogenin 10 (**13**, 68 mg), prosapogenin 11 (**14**, 29 mg), and prosapogenin 12 (**15**, 21 mg).

Prosapogenin 9 (12): a white amorphous powder, $[\alpha]_D$ −14.6° (*c* 0.40, MeOH); ^{1}H-NMR (in pyridine-d_5) δ 0.99, 1.00, 1.05, 1.08, 1.18, 1.33, 1.56 (each 3H, s, *tert*-methyl), 1.49 (3H, d, J = 6.6 Hz, fuc − 6), 1.60 (3H, d, J = 5.1 Hz, qui − 6), 1.77 (3H, d, J = 6.2 Hz, rha − 6), 1.85 [3H, s, monoterpenoid acid (MTA)-10], 1.91 (3H, s, MTA-9), 4.88 (1H, d, J = 7.7 Hz, qui − 1), 4.90 (1H, d, J = 8.8 Hz, glc − 1), 4.92 (1H, d, J = 8.1 Hz, fuc − 1),

5.00 (1H, d, J = 7.7 Hz, xyl − 1), 5.33 (1H, d, J = 7.7 Hz, glc‴ − 1), 5.64 (1H, br s, H-12), 5.92 (1H, br s, rha − 1), 6.06 (1H, d, J = 7.7 Hz, glc″ − 1), 6.23 (1H, d, J = 6.6 Hz, ara(f) − 1); ^{13}C-NMR (in pyridine-d_5) data, see Table 2; negative-ion FABMS m/z [M − H]$^-$ 1841, [M − H − ara(f)]$^-$ 1709, [M − H − glc]$^-$ 1679, [M − H − 602]$^-$ 1239.

Prosapogenin 10 (13): a white amorphous powder; $[\alpha]_D$ −13.6° (c 0.51, MeOH); ^{1}H-NMR (in pyridine-d_5) δ 0.99, 1.04, 1.05, 1.10, 1.18, 1.33, 1.52 (each 3H, s, *tert*-methyl), 1.49 (3H, d, J = 6.2 Hz, fuc − 6), 1.60 (3H, d, J = 5.1 Hz, qui − 6), 1.77 (3H, d, J = 5.5 Hz, rha − 6), 1.91 (3H, s, MTA-10), 4.86 (1H, d, J = 7.7 Hz, qui − 1), 4.92 (1H, d, J = 7.7 Hz, glc − 1), 5.01 (1H, d, J = 8.1 Hz, fuc − 1), 5.09 (1H, d, J = 7.0 Hz, xyl − 1), 5.33 (1H, d, J = 7.7 Hz, glc‴ − 1), 6.06 (1H, d, J = 8.1 Hz, glc″ − 1), 6.20 (1H, d, J = 7.0 Hz, ara(f) − 1); ^{13}C-NMR (pyridine-d_5) data, see Table 2; negative-ion FABMS m/z [M − H]$^-$ 1857, [M − H − glc]$^-$ 1695, [M − H − 602]$^-$ 1255, [M − H − 602 − qui − MTA]$^-$ 927, [M − H − 602 − qui − MTA − ara(f)]$^-$ 797.

Prosapogenin 11 (14): a white amorphous powder; $[\alpha]_D$ −6.5° (c 0.47, MeOH); ^{1}H-NMR (in pyridine-d_5) δ 0.93, 0.97 × 2, 1.09, 1.18, 1.35, 1.41 (each 3H, s, *tert*-methyl), 1.49 (3H, d, J = 6.2 Hz, fuc − 6), 1.57 (3H, s, MTA-10), 1.61 (3H, d, J = 5.1 Hz, qui − 6), 1.81 (3H, d, J = 5.5 Hz, rha − 6), 1.86 (3H, s, MTA-9), 4.90 (1H, d, J = 8.1 Hz, qui − 1), 5.04 (1H, d, J = 7.7 Hz, glc − 1), 5.06 (1H, d, J = 6.6 Hz, fuc − 1), 5.07 (1H, d, J = 6.6 Hz, xyl − 1), 5.35 (1H, d, J = 7.7 Hz, glc‴ − 1), 6.10 (1H, d, J = 7.7 Hz, glc″ − 1), 6.24 (1H, d, J = 6.6 Hz, ara(f) − 1); ^{13}C-NMR (pyridine-d_5) data, see Table 2 ; negative-ion FABMS m/z [M − H]$^-$ 1825, [M − H − glc]$^-$ 1663, [M − H − 602]$^-$ 1223.

Prosapogenin 12 (15): a white amorphous powder; $[\alpha]_D$ −22.3° (c 0.49, MeOH); ^{1}H-NMR (in pyridine-d_5) δ 0.93, 0.98, 0.99, 1.17, 1.21, 1.31, 1.38 (each 3H, s, *tert*-methyl), 1.50 (3H, d, J = 6.2 Hz, fuc − 6), 1.57 (3H, s, MTA-10), 1.60 (3H, d, J = 5.1 Hz, qui − 6), 1.80 (3H, d, J = 5.5 Hz, rha − 6), 1.86 (3H, s, MTA-9), 4.89 (1H, d, J = 7.7 Hz, qui − 1), 5.00 (1H, d, J = 7.7 Hz, glc − 1), 5.02 (1H, d, J = 6.6 Hz, fuc − 1), 5.04 (1H, d, J = 7.0 Hz, xyl − 1), 5.34 (1H, d, J = 7.7 Hz, glc‴ − 1), 5.40 (1H, d, J = 7.3 Hz, glc′ − 1), 6.02 (1H, br s, rha − 1), 6.09 (1H, d, J = 7.7 Hz, glc″ − 1), 6.24 (1H, d, J = 8.1 Hz, ara(f) − 1); ^{13}C-NMR (pyridine-d_5) data, see Table 2; negative-ion FABMS m/z [M − H]$^-$ 1987, [M − H − glc]$^-$ 1825, [M − H − 602]$^-$ 1385, [M − H − 602 − glc]$^-$ 1223.

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