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Kinmoonosides A−C, Three New Cytotoxic Saponins from the Fruits of *Acacia concinna*, a Medicinal Plant Collected in Myanmar

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Three genuine saponins, named kinmoonosides A−C (**1**−**3**), have been isolated, together with a new monoterpenoid (**4**), from a methanolic extract of the fruits of *Acacia concinna*. The structures of kinmoonosides A−C were elucidated on the basis of spectral analysis as 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-{(6*R*,2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2′*E*)-6′-hydroxyl-2′-hydroxymethyl-6′-methyl-2′,7′-octadienoyl-β-D-quinovopyranosyl]-2,7-octadienoyl}acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (**1**); 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-{(6*S*,2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2′*E*)-6′-hydroxyl-2′-hydroxymethyl-6′-methyl-2′,7′-octadienoyl-β-D-quinobopyranosyl]-2,7-octadienoyl}acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (**2**); and 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-[(2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl]acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester (**3**), respectively. The new monoterpenoid **4** was determined as 4-*O*-[(2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl]-D-quinovopyranose. Compounds **1**−**3** showed significant cytotoxicity against human HT-1080 fibrosarcoma cells.

Acacia concinna Wall. (Leguminosae) is a medicinal plant that grows in tropical rainforests of southern Asia, and the fruits of this plant are used for washing hair, for promoting hair growth, as an expectorant, emetic, and purgative.[1] Although the pods of this plant are known to contain several saponins based on acacic acid,[2] previous chemical examinations only resulted in the identification of flavonoids[1] and monoterpenoids.[3] The structures of several triterpene genins and prosapogenins were determined,[4−6] and the genuine saponins were postulated to have structures similar to the leguminous saponins, acasiasides A and B from *Acacia auriculiformis* and julibrosides I−III from *Albizia julibrissin*.[7]

In the course of our study on medicinal plants from southeast Asia,[8] we have found recently that a MeOH extract of the fruits of *A. concinna* from Myanmar showed significant cytotoxicity against human HT-1080 fibrosarcoma cells. Thus, we examined the constituents and identified three new genuine saponins, which were given the trivial names kinmoonosides A−C (**1**−**3**), based on a local name in Myanmar of the crude drug, "Kin moon thee". Also isolated was a new monoterpenoid (**4**). In this paper, we report the isolation and structure elucidation of **1**−**4** by spectroscopic techniques. Compounds **1**−**3** were found to be significantly cytotoxic against human HT-1080 fibrosarcoma cells.

Results and Discussion

The pods of *A. concinna* were extracted successively with $CHCl_3$, EtOAc, MeOH, MeOH−H_2O (1:1), and H_2O, and the cytotoxicity of each extract was tested against the highly liver-metastatic murine colon 26-L5 carcinoma[9] and the human HT-1080 fibrosarcoma[10] cell lines. All extracts showed only weak cytotoxicity against colon 26-L5 carcinoma cells; but against human HT-1080 fibrosarcoma cells, they showed cytotoxicities of various potencies [ED_{50}: $CHCl_3$ and EtOAc extracts, > 100 μg/mL; MeOH extract, 14.0 μg/mL; MeOH−H_2O (1:1) extract, 21.9 μg/mL; H_2O extract, 56.2 μg/mL]. The MeOH extract, which showed the strongest activity, was separated into four fractions by Diaion HP-20 column chromatography. The 75% MeOH−H_2O eluate, which showed the most potent cytotoxicity

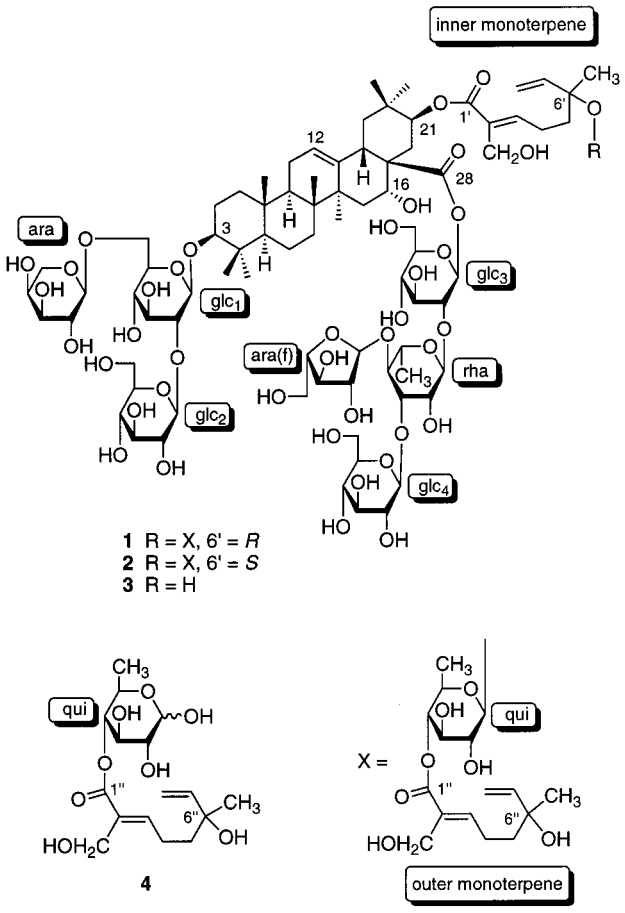

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Table 1. ^{13}C NMR Data in CD_3OD for the Aglycon Moieties of Kinmoonosides A−C (**1**−**3**) and the New Prosapogenins **9** and **10**

carbon	1	2	3	9	10
1	39.9	39.9	39.9	39.8	39.7
2	27.3	27.2	27.3	27.1	26.9
3	91.4	91.4	91.4	91.4	91.0
4	40.5	40.5	40.5	40.4	39.9
5	57.1	57.1	57.1	57.1	56.9
6	19.6	19.6	19.5	19.3	19.3
7	34.8	34.5	34.6	34.3	34.3
8	40.8	40.8	40.8	40.6	40.5
9	48.0	48.0	48.0	48.1	48.0
10	37.9	37.9	37.9	37.9	37.9
11	24.3	24.4	24.3	24.5	24.5
12	124.1	124.0	123.3	123.5	123.7
13	143.7	143.7	143.7	144.6	144.3
14	42.7	42.6	42.7	42.5	42.5
15	36.2	36.2	36.2	36.0	35.9
16	74.2	74.2	74.2	74.9	74.9
17	52.3	52.3	52.3	52.5	52.2
18	41.7	41.6	41.7	41.6	41.4
19	48.5	48.5	48.5	49.0	49.1
20	35.9	35.9	35.9	36.7	36.7
21	78.7	78.7	78.7	75.2	74.7
22	36.4	36.4	36.4	41.1	40.9
23	28.5	28.5	28.5	29.8	29.7
24	17.0	17.0	17.0	16.9	17.1
25	16.2	16.2	16.2	16.1	16.0
26	17.8	17.8	17.7	17.9	17.8
27	27.3	27.3	27.3	27.3	27.3
28	175.3	175.3	175.3	179.0	173.3
29	29.4	29.4	29.4	28.5	28.6
30	19.4	19.4	19.4	18.1	18.1

(ED_{50}: H_2O eluate, 17.9 μg/mL; 50% MeOH−H_2O eluate, 21.5 μg/mL; 75% MeOH−H_2O eluate, 2.1 μg/mL; MeOH eluate, 10.0 μg/mL), gave three genuine saponins named kinmoonosides A−C (**1**−**3**), while the 50% MeOH−H_2O eluate yielded the new monoterpenoid **4**.

The monoterpenoid **4** was obtained as a pale-yellow oil, and its molecular formula was determined to be $C_{16}H_{26}O_8$ by HRFABMS. Its ^1H and ^{13}C NMR spectra (Table 3) were similar to those of methyl (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoate 6-*O*-β-D-quinovoside, previously isolated from an alkaline hydrolysate of a saponin fraction of *A. concinna* collected in Bangladesh.[7] However, the signals for the quinovose unit appeared as a pair of two anomers (1:1), and the signal for the quinovose (qui) H-4 appeared at low field (α-anomer, δ_H 4.67; β-anomer, δ_H 4.62). Thus, the octadienoic acid moiety was considered to be located at C-4, not at C-1, of the quinovose, which was confirmed by the HMBC correlation between H-4 of the quinovose (qui) and C-1″ of the acid. From these data, the monoterpenoid **4** was concluded to be 4-*O*-[(2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl]-D-quinovopyranose, except for the stereochemistry at C-6″ of the octadienoic acid, which could not be determined due to the small amount obtained.

To elucidate the structures of the genuine saponins, we first conducted the alkaline hydrolysis of the crude saponin fraction of *A. concinna* and obtained four monoterpenoids (**5**−**8**) and five prosapogenins (**9**−**13**), while acid hydrolysis of the alkaline hydrolysate gave acacic acid lactone (**14**)[5] (Figure 1). By analysis of their spectral data and comparison with literature values, monoterpenoids **5**−**7** were determined to be known compounds, namely, menthiafolic acid (**5**),[7] a mixture (1:1) of (6*S*)- and (6*R*)-menthiafolic acid 6-*O*-β-D-quinovopyranoside (**6**),[7] and a mixture (2:1) of two diastereomers of 3,6-epoxy-2-hydroxymethyl-6-methyl-7-octenoic acid (**7**),[11] while prosapogenins **11**−**13** were identified as 3-*O*-β-D-xylopyranosyl(1→2)-α-L-fucopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranoside (**11**);[12] acacic acid 3-*O*-β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**12**);[13] and acacic acid lactone 3-*O*-β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranoside (**13**, concinnoside F = albiziasaponin C),[5,14] respectively. Among the new compounds, monoterpenoid **8** was determined as a mixture (1:1) of (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside, by analysis of the spectral data and derivatization to methyl (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoate 6-*O*-β-D-quinovopyranoside[6] and then to methyl (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoate.[6] The absolute configuration at C-6 of each isomer was determined to be *R* (**8a**) and *S* (**8b**) by comparing the ^1H and ^{13}C NMR data (Table 3) with those of **6** and its methyl ester.[7] The new prosapogenins, on the other hand, showed similar ^1H and ^{13}C NMR data (Tables 1 and 2) to those of prosapogenins **12** and **13**, and to concinnosides A−F,[5] prosapogenins of *A. concinna* collected in Bangladesh, and their structures were determined by spectral data (Figure 2 in Supporting Information) interpretation to be acacic acid 3-*O*-α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranoside (**9**) and acacic acid 3-*O*-α-L-arabinopyranosyl(1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**10**).

Kinmoonoside A (**1**) was obtained as a colorless amorphous solid, and its molecular formula was determined to be $C_{96}H_{152}O_{47}$ by HRFABMS. By extensive analysis of its 2D NMR spectra, the ^1H and ^{13}C NMR data for the aglycon moiety and the trisaccharide unit at C-3 were found to be almost the same as those of prosapogenin **9** (Tables 1 and 2). Thus, **1** was found to be a derivative of 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}acacic acid, which was confirmed by the long-range correlations observed in the HMBC spectrum (Figure 3 in Supporting Information). In addition, the ^1H and ^{13}C NMR spectra of **1** indicated the presence of five additional sugars and two monoterpenes (Tables 2 and 3). Based on the chemical shifts and coupling constants, the sugars were determined to be two β-glucopyranose units (glc_3-1: δ_H 5.33, d, J = 7.5 Hz, δ_C 95.4; glc_4-1: δ_H 4.49, d, J = 7.7 Hz, δ_C 105.8), an α-rhamnopyranose (rha-1: δ_H 5.34, br s; δ_C 101.3), an α-arabinofuranose [ara(f)-1: δ_H 5.35, br s; δ_C 111.0], and a β-quinovopyranose (qui-1: δ_H 4.40, d, J = 7.7 Hz; δ_C 99.2), while the monoterpenes were two (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid units.

The two octadienoic acid moieties were placed at C-21 of the aglycon and at C-4 of the quinovose (qui), from the lowfield shifts of H-21 (δ_H 5.50) of the aglycon and H-4 (δ_H 4.64) of the quinovose (qui) and acylation shifts[15,16] at C-20 (**1**, δ_C 35.9; **9**, δ_C 36.7), C-21 (**1**, δ_C 78.7; **9**, δ_C 75.2), and C-22 (**1**, δ_C 36.4; **9**, δ_C 41.1) of the aglycon and at C-3 (**1**, δ_C 75.6; **8a**, δ_C 77.9), C-4 (**1**, δ_C 77.7; **8a**, δ_C 77.0), and C-5 (**1**, δ_C 70.9; **8a**, δ_C 72.8) of the quinovose (qui). From these data and analysis of the HMBC correlations (Figure 3 in Supporting Information), the ester residue at C-21 of the aglycon was determined as (2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2′*E*)-6′-hydroxyl-2′-hydroxymethyl-6′-methyl-2′,7′-octadienoyl-β-D-quinovopyranosyl]-2,7-octadienoate, which was supported by the observation of ^1H and ^{13}C NMR data similar to those of monoterpenoids **4** and **8** (Table 3). The NMR data, especially for H-7′, H-10′, C-5′, and C-8′ of the inner monoterpene, more closely resembled those of **8a** (6*R*) rather than **8b** (6*S*), indicating the absolute configuration at C-6′ of the inner monoterpene to be *R*, but that

Table 2. ^{13}C NMR Data in CD_3OD for Sugar Moieties of Kinmoonosides A−C (**1**−**3**) and the New Prosapogenins **9** and **10**

carbon	**1**	**2**	**3**	**9**	**10**
glc_1					glcNAc
1	105.3 (4.44, d, 7.5)[a]	105.3 (4.44, d, 7.0)[a]	105.4 (4.44, d, 7.3)[a]	105.3 (4.47, d, 7.5)[a]	104.9 (4.44, d, 8.8)[a]
2	81.3	81.2	81.2	81.1	57.7
3	78.3	78.2	78.3	78.3	75.6
4	71.3	71.2	71.3	71.6	72.0
5	76.7	76.6	76.7	76.6	76.5
6	69.6	69.6	69.6	69.6	69.5
CH_3CO					23.1, 180.2
glc_2					
1	104.6 (4.65, d, 7.6)[a]	104.6 (4.66, d, 7.7)[a]	104.6 (4.44, d, 7.6)[a]	104.5 (4.67, d, 8.0)[a]	
2	76.3	76.3	76.3	76.3	
3	77.9	77.8	77.9	77.8	
4	71.9	72.0	71.9	71.9	
5	78.3	78.2	78.3	78.3	
6	63.0	63.0	63.0	63.1	
ara					
1	105.0 (4.33, d, 6.5)[a]	105.0 (4.33, d, 6.2)[a]	105.0 (4.34, d, 6.6)[a]	105.0 (4.33, d, 6.2)[a]	104.9 (4.34, d, 6.6)[a]
2	72.4	72.4	72.4	72.3	72.3
3	74.2	74.1	74.2	74.1	74.0
4	69.1	69.1	69.1	69.3	69.2
5	66.4	66.4	66.5	66.5	66.4
glc_3					
1	95.4 (5.33, d, 7.5)[a]	95.3 (5.33, d, 7.4)[a]	95.4 (5.33, d, 7.3)[a]		
2	76.3	76.4	76.3		
3	79.0	79.0	79.0		
4	71.1	71.1	71.1		
5	77.6	77.6	77.7		
6	62.2	62.2	62.2		
rha					
1	101.3 (5.34, br s)[a]	101.3 (5.34, br s)[a]	101.3 (5.34, br s)[a]		
2	71.4	71.4	71.4		
3	82.7	82.7	82.7		
4	78.9	78.6	78.6		
5	69.3	69.3	69.3		
6	18.6	18.6	18.6		
ara(f)					
1	111.0 (5.35, br s)[a]	111.0 (5.35, br s)[a]	111.0 (5.36, br s)[a]		
2	83.9	83.9	83.9		
3	78.5	78.5	78.6		
4	85.6	85.5	85.5		
5	63.1	63.1	63.1		
glc_4					
1	105.8 (4.49, d, 7.7)[a]	105.8 (4.49, d, 7.7)[a]	105.8 (4.50, d, 7.8)[a]		
2	75.3	75.5	75.4		
3	78.1	78.1	78.1		
4	71.9	71.6	71.6		
5	78.5	78.6	78.6		
6	62.3	62.3	62.3		

[a] Chemical shift, coupling pattern, and coupling constant of the corresponding anomeric proton.

of the outer monoterpene could not be determined due to shortage of the amount obtained.

The remaining residues to be placed were two β-glucopyranoses, an α-arabinofuranose, and an α-rhamnopyranose. The FABMS of **1** revealed a fragment ion at *m*/*z* 1477 $[M + Na - 603]^+$, suggesting that the four sugars were linked at C-28 as a tetrasaccharide residue.[12,17] The characteristic signals for the anomeric proton (δ_H 5.33) and carbon (δ_C 95.4) of the β-glucopyranose (glc_3) suggested that this glucose should be directly attached to C-28 through an ester bond, which was confirmed by the HMBC correlation between H-1 (δ_H 5.33) of the glucose (glc_3) and C-28 (δ_C 175.3) of the aglycon. Similarly, long-range correlations were observed between C-2 (δ_C 76.3) of the glucose (glc_3) and H-1 (δ_H 5.34) of the rhamnose (rha), between C-3 (δ_C 82.7) of the rhamnose (rha) and H-1 (δ_H 4.99) of the glucose (glc_4), and between C-4 (δ_C 78.9) of the rhamnose (rha) and H-1 (δ_H 5.35) of the arabinose [ara(f)] (Figure 3 in Supporting Information). Thus, the tetrasaccharide residue at C-28 of the aglycon was determined to be α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl.

From these data, kinmoonoside A was assigned as 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-{(6*R*,2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2′*E*)-6′-hydroxyl-2′-hydroxymethyl-6′-methyl-2′,7′-octadienoyl-β-D-quinobopyranosyl]-2,7-octadienoyl}acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (**1**). Previously, several triterpenoid saponins with a menthiafolic acid and/or (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid residue have been reported.[16,18] The acid moiety, however, usually has a 6*S* configuration, and only three triterpenoid saponins with a (6*R*)-acid are known, proceraoside C from *Albizia procera* and julibrosides J_1 and J_{14} from *A. julibrissin* (Leguminosae).[19] However, the acid is menthiafolic acid, and there is no prior example of a triterpenoid saponin with a (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid unit having a (6*R*)-configuration.

Kinmoonoside B (**2**) gave a $[M + H]^+$ ion at *m*/*z* 2057.9575 in its HRFABMS, suggesting the same molecular formula, $C_{96}H_{152}O_{47}$, as **1**. Its ^1H and ^{13}C NMR data (Tables 1−3 and Experimental Section), analyzed by 2D

Table 3. ^{1}H and ^{13}C NMR Data in CD_3OD for the C-21 Saccharide Portions of Kinmoonosides A−C (**1**−**3**) and Monoterpenoids **4** and **8**

	1		**2**		**3**		**4**				**8a**		**8b**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H		δ_C		δ_H	δ_C	δ_H	δ_C
inner monoterpene														
1′		168.7		168.7		168.7						171.9		171.9
2′		133.0		132.9		133.0						133.4		133.4
3′	6.91 br t (7.7)	148.0	6.91 br t (7.7)	148.0	6.91) br t (7.8)	147.9					6.85 br t (6.6)	146.6	6.85 br t (6.6)	146.5
4′	2.54 m	24.2	2.50 m	24.2	2.35 m	24.5					2.40 m	24.0	2.40 m	24.1
5′	1.79 m	39.5	1.75 m	41.3	1.64 m	42.0					1.73 m	39.9	1.73 m	41.5
6′		80.9		81.0		73.7						80.8		80.9
7′	6.03 dd (17.5, 10.8)	144.2	5.96 dd (17.5, 10.9)	144.0	5.91 dd (17.2, 10.7)	145.9					6.03 dd (17.3, 11.0)	144.3	5.94 dd (17.3, 11.0)	144.1
8′	5.26 dd (17.5, 1.5)	115.3	5.25 dd (17.5, 1.5)	116.0	5.23 dd (17.2, 1.5)	112.5					5.16 br d (11.0)	115.2	5.22 br d (10.7)	116.0
	5.18 dd (10.8, 1.5)		5.21 dd (10.9, 1.5)		5.05 dd (10.7, 1.5)						5.22 br d (17.3)		5.28 br d (17.1)	
9′	4.33 m	56.7	4.32 m	56.6	4.32 m	56.6					4.31 br s	57.2	4.31 br s	57.2
10′	1.35 s	24.5	1.39 s	23.8	1.28 s	27.9					1.34 s	24.1	1.37 s	23.8
qui							α-anomer	β-anomer	α-anomer	β-anomer				
1	4.40 d (7.7)	99.2	4.40 d (7.7)	99.3			5.07 br s	4.50 d (7.8)	93.8	98.1	4.31 d (7.8)	99.1	4.35 d (7.8)	99.3
2	3.24 m	75.3	3.24 m	75.3			3.45 dd (9.5, 3.9)	3.22 dd (9.3, 7.8)	74.1	76.6	3.16 dd (9.1, 7.8)	75.3	3.16 dd (9.1, 7.8)	75.4
3	3.58 m	75.6	3.58 m	75.6			3.83 t (9.5)	3.55 t (9.3)	72.5	75.6	3.28 t (9.1)	77.9	3.28 t (9.1)	77.9
4	4.64 t (9.4)	77.7	4.64 t (9.4)	77.6			4.62 t (9.5)	4.67 t (9.3)	78.8	77.7	2.98 t (9.1)	77.0	2.98 t (9.1)	77.0
5	3.50 m	70.9	3.50 m	70.9			4.05 m	3.57 m	66.2	71.2	3.18 m	72.8	3.18 m	72.9
6	1.12 d (6.0)	18.3	1.12 d (6.0)	18.3			1.09 d (6.4)	1.15 d (6.4)	18.1	18.1	1.23 d (5.9)	18.3	1.22 d (5.3)	18.3
outer monoterpene														
1″		168.2		168.2			6.95 br t (8.0)		168.2					
2″		132.5		132.5			2.40 m		125.2	132.5				
3″	6.94 br t (7.7)	148.5	6.95 br t (7.7)	148.5			1.63 m		148.4					
4″	2.37 m	24.5	2.37 m	24.4					24.5					
5″	1.65 m	41.9	1.64 m	41.9			5.92 dd (17.4, 11.0)		41.9					
6″		73.6		73.6			5.06 dd (11.0, 1.2)		73.6					
7″	5.91 dd (17.4, 10.9)	145.9	5.91 dd (17.3, 10.9)	145.9			5.23 dd (17.4, 1.2)		145.9					
8″	5.23 dd (17.3, 1.7)	112.5	5.22 dd (17.3, 1.5)	112.5			4.33 br s		112.5					
	5.06 dd (10.9, 1.7)		5.06 dd (10.9, 1.5)				1.27 s							
9″	4.33 m	56.5	4.32 m	56.5					56.5					
10″	1.28 s	27.9	1.28 s	27.9					27.9					

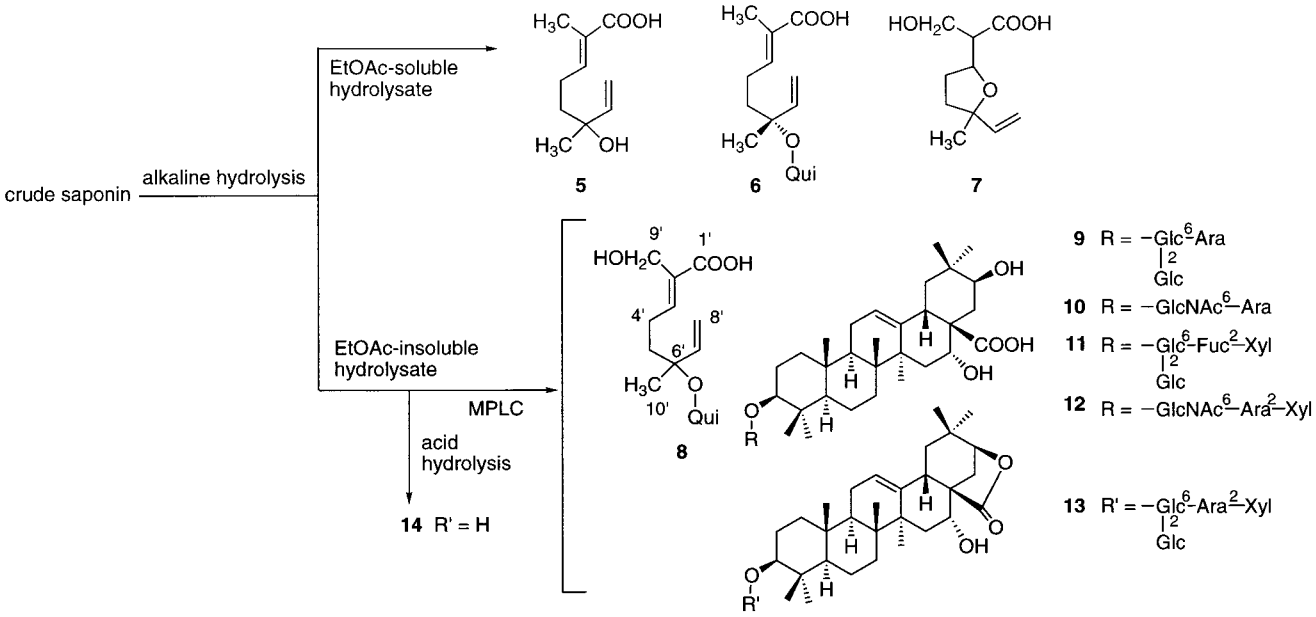

Figure 1. Hydrolysis of the Crude Saponin Mixture of *A. concinna*.

NMR spectra, were very similar to those of **1**, except for those of C-5′, C-7′, C-8′, and C-10′ of the inner monoterpene. This difference was reasonably understood to be due to the configurational difference at C-6′ of the inner monoterpene, as observed in **8a** (6′*R*) and **8b** (6′*S*). Thus, kinmoonoside B was concluded to be 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-{(6*S*,2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2′*E*)-6′-hydroxyl-2′-hydroxymethyl-6′-methyl-2′,7′-octadienoyl-β-D-quinobopyranosyl]-2,7-octadienoyl}acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (**2**).

Kinmoonoside C (**3**) was determined to have the molecular formula $C_{80}H_{128}O_{40}$ by HRFABMS, and its ^1H and ^{13}C NMR spectra (Tables 1−3 and Experimental Section) resembled those of kinmoonosides A (**1**) and B (**2**). However, they revealed a lack of the outer monoterpene and the β-quinovopyranose moiety in **1** and **2**. Thus, kinmoonoside C was concluded to be 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-[(2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl]-acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (**3**), which was confirmed by similar HMBC correlations observed for **1**.

Finally, the cytotoxic activity of compounds **1**−**13** was examined against human HT-1080 fibrosarcoma cells. Although monoterpenoids **4**−**8** and prosapogenins **9**−**13** were inactive (ED_{50}, >100 μg/mL), the genuine saponins **1**−**3** showed significant cytotoxic activity, with ED_{50} values of 0.70, 0.91, and 2.83 μM, respectively. Their cytotoxicity was greater than that of 5-fluorouracil[20] (ED_{50}, 8.0 μM). ED_{50} values of < 4 μg/mL are regarded as being significantly cytotoxic.[21] By comparing the cytotoxicity of the genuine saponins **1**−**3** with the prosapogenins **9**−**13**, the tetrasaccharide at C-28 of the aglycon may be considered to be important for the mediation of their cytotoxicity, as reported in the case of julibrosides I−III.[13] Although both the trisaccharide unit at C-3 of the aglycon and the ester moiety at C-21 of the aglycon were not crucial for the cytotoxicity, difference in the activities among **1**−**3** seems to suggest that the ester at C-21 of the aglycon may intensify the cytotoxicity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. ^1H, ^{13}C, and 2D NMR were taken on a JEOL GX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. FABMS and HRFABMS measurements were carried out on a JEOL JMS-700T spectrometer, and glycerol was used as a matrix. Column chromatography was performed with Diaion HP-20 or MCI-gel CHP-20P (both Mitsubishi Kasei Co., Ltd., Tokyo, Japan), and analytical and preparative TLC were carried out on precoated Merck Kieselgel 60F_{254} plates (0.25 or 0.50 mm thickness) or RP_{18} F_{254} plates (0.25 mm thickness). Medium-pressure liquid chromatography (MPLC) was performed with a Chemco model 61-M-2 system, and HPLC was conducted with a Shimadzu LC-6AD system.

Plant Material. Fruits of *A. concinna* were purchased from Sandhi Brothers Trading Co., Ltd., at Yangon, Myanmar, in October 1997, and identified by Prof. Daw Khin May Ni (University of Distance Education Mandalay, Myanmar). A voucher sample (TMPW no. 18579) is preserved in the Museum for Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.

Extraction and Isolation. Fruits of *A. concinna* were separated into pods and seeds, and the pods (3.6 kg) were extracted successively at room temperature with $CHCl_3$ (5 L, 7 d × 2), EtOAc (5 L, 7 d × 2), and MeOH, (5 L, 7 d × 2), and then with MeOH−H_2O (1:1) (5 L, reflux × 2) and H_2O (5 L, reflux × 2). Each extract was evaporated under reduced pressure to give $CHCl_3$ (20 g), EtOAc (19 g), MeOH (1.2 kg), MeOH−H_2O (1:1) (276 g), and H_2O (208 g) residues. The MeOH extract (300 g) was subjected to Diaion HP-20 (1.5 L) column chromatography with MeOH−H_2O (0%, 50%, 75%, 100%; each 3 L) to give four fractions (H_2O eluate, 161 g; 50% MeOH−H_2O eluate, 17 g; 75% MeOH−H_2O eluate, 49 g; MeOH eluate, 31 g).

A part (3.0 g) of the 50% H_2O−MeOH eluate was separated by MCI-gel CHP-20P (50 mL) column chromatography with a MeOH−H_2O (10%, 25%, 45%, 60%, 80%, 100%) solvent system to give six fractions (10% MeOH−H_2O eluate, 240 mg; 25% MeOH−H_2O eluate, 428 mg; 45% MeOH−H_2O eluate, 702 mg; 60% MeOH−H_2O eluate, 520 mg; 80% MeOH−H_2O eluate, 664 mg; MeOH eluate, 250 mg). The 25% MeOH−H_2O eluate (400 mg) was subjected to MPLC separation with $CHCl_3$−MeOH−H_2O (7:3:0.5) to give five fractions (fraction 1, 48 mg; fraction 2, 60 mg; fraction 3, 110 mg; fraction 4, 45 mg; fraction 5, 80 mg), and fraction 3 gave 4-*O*-[(2*E*)-6-hydroxyl-2-hydroxy-

methyl-6-methyl-2,7-octadienoyl]-D-quinovopyranose (**4**, 12.3 mg) by preparative TLC with $CHCl_3$−MeOH−H_2O (7:3:0.5).

The 75% MeOH−H_2O eluate was separated (500 mg each, 6 times) by MPLC (column: Lichroprep RP_{18}, 40−63 μm, size B) with a CH_3CN−H_2O solvent system (20−40% at intervals of 5%; each 200 mL; flow rate, 2.0 mL/min) to give five fractions (fraction 1, 760 mg; fraction 2, 870 mg; fraction 3, 494 mg; fraction 4, 792 mg; fraction 5, 57 mg). Fraction 1 (600 mg) was again separated by MPLC (column: Lichroprep RP_8, 40−63 μm, size A) with a CH_3CN−H_2O solvent system (20−35% at intervals of 3%; each 50 mL; flow rate, 2.0 mL/min) to yield six fractions, and subfraction 2 (67 mg) was subjected to reversed-phase preparative TLC with Me_2CO−H_2O (4:6) to give kinmoonoside C (**3**, 18.6 mg). In turn, fraction 2 (870 mg) was separated by MPLC (column: Lichroprep RP_{18}, 40−63 μm, size B) with an Me_2CO−H_2O solvent system (20−40% at intervals of 2%; each 100 mL; flow rate, 2.0 mL/min) to yield 20 fractions, and subfractions 13 (106 mg) and 16 (76 mg) were subjected to an HPLC separation (column: Supelco Discovery C_{18}, 250 mm × 21.2 mm, 5 μm; UV, 220 nm) with CH_3CN−H_2O (27.5:72.5 and 28:72, respectively; flow rate, 10 mL/min) to give kinmoonosides A (**1**, 13.1 mg) and B (**2**, 13.3 mg).

Kinmoonoside A (1): colorless amorphous solid; $[\alpha]^{25}_D$ −14.4° (*c* 0.44, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) aglycon δ 5.50 (1H, dd, J = 10.9, 5.6 Hz, H-21), 5.35 (1H, br s, H-12), 4.49 (1H, br s, H-16), 3.24 (1H, m, H-3), 2.98 (1H, m, H-18), 1.42 (3H, s, H_3-27), 1.09 (3H, s, H_3-24), 1.05 (3H, s, H_3-30), 0.97 (3H, s, H_3-25), 0.88 (3H, s, H_3-29), 0.86 (3H, s, H_3-23), 0.78 (3H, s, H_3-26); for other NMR data, see Tables 1−3; FABMS *m/z* 2080.0 $[M + Na]^+$, 2058.0 $[M + H]^+$, 1477.7 [M + Na − glc − ara − rha − glc]$^+$; HRFABMS *m/z* 2057.9541 (calcd for $C_{96}H_{153}O_{47}$ $[M + H]^+$, 2057.9582).

Kinmoonoside B (2): colorless amorphous solid; $[\alpha]^{25}_D$ −38.5° (*c* 0.66, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) aglycon δ 5.49 (1H, dd, J = 10.4, 5.2 Hz, H-21), 5.35 (1H, br s, H-12), 4.48 (1H, br s, H-16), 3.22 (1H, m, H-3), 2.98 (1H, m, H-18), 1.42 (3H, s, H_3-27), 1.09 (3H, s, H_3-24), 1.05 (3H, s, H_3-30), 0.97 (3H, s, H_3-25), 0.88 (3H, s, H_3-29), 0.86 (3H, s, H_3-23), 0.78 (3H, s, H_3-26); for other NMR data, see Tables 1−3; FABMS *m/z* 2080.0 $[M + Na]^+$, 2058.0 $[M + H]^+$, 1477.7 [M + Na − glc − ara − rha − glc]$^+$; HRFABMS *m/z* 2057.9575 (calcd for $C_{96}H_{153}O_{47}$ $[M + H]^+$, 2057.9582).

Kinmoonoside C (3): colorless amorphous solid; $[\alpha]^{25}_D$ +8.4° (*c* 0.35, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) aglycon δ 5.49 (1H, dd, J = 10.5, 5.1 Hz, H-21), 5.36 (1H, br s, H-12), 4.49 (1H, br s, H-16), 3.22 (1H, m, H-3), 3.08 (1H, m, H-18), 1.42 (3H, s, H_3-27), 1.09 (3H, s, H_3-24), 1.05 (3H, s, H_3-30), 0.97 (3H, s, H_3-25), 0.88 (3H, s, H_3-29), 0.87 (3H, s, H_3-23), 0.78 (3H, s, H_3-26); for other NMR data, see Tables 1−3; FABMS *m/z* 1751.8 $[M + Na]^+$, 1729.8 $[M + H]^+$; HRFABMS *m/z* 1751.7849 (calcd for $C_{80}H_{128}O_{40}Na$ $[M + Na]^+$, 1751.7880), 1729.8029 (calcd for $C_{80}H_{129}O_{40}$ $[M + H]^+$, 1729.8060).

4-*O*-[(2*E*)-6-Hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl]-D-quinovopyranose (4): light yellow oil; $[\alpha]^{25}_D$ +19.3° (*c* 0.40, MeOH); ^{1}H (CD_3OD, 400 MHz) and ^{13}C (CD_3OD, 100 MHz) NMR, see Table 3; FABMS *m/z* 369.2 $[M + Na]^+$, 347.2 $[M + H]^+$; HRFABMS *m/z* 347.1734 (calcd for $C_{16}H_{27}O_8$ $[M + H]^+$, 347.1706).

Hydrolysis of Saponin Fraction. A part of the 75% MeOH−H_2O eluate (10.0 g) was suspended in BuOH (100 mL), and the soluble material (200 mg) was separated off by filtration. The BuOH-insoluble fraction (9.8 g) was hydrolyzed with 0.5 M NaOH−MeOH (7:1, 160 mL) at room temperature for 24 h. After neutralization by 1 M HCl, followed by concentration in vacuo to remove MeOH, the residual solution was extracted with EtOAc (200 mL × 3) and then BuOH (200 mL × 3) to give EtOAc-soluble (1.5 g) and BuOH-soluble (5.3 g) hydrolysates.

The EtOAc-soluble hydrolysate (500 mg) was subjected to MPLC (column: Lichroprep Si60, 40−63 μm, size B; solvent: $CHCl_3$−MeOH−H_2O, 8:4:0.5; flow rate: 2.0 mL/min) to give five fractions (fraction 1, 33 mg; fraction 2, 100 mg; fraction 3, 161 mg; fraction 4, 139 mg; fraction 5, 70 mg). Fractions 2−4 were subjected separately to preparative TLC with $CHCl_3$−MeOH−H_2O−HOAc (8:2:0.5:0.01, 8:3:0.5:0.01, and 8:4:0.5:0.01, respectively) to give menthiafolic acid[7] (**5**, 7.0 mg), a mixture (1:1) of (6*S*)- and (6*R*)-menthiafolic acid 6-*O*-β-D-quinovopyranoside[7] (**6**, 60.0 mg), and a mixture (2:1) of two diastereomers of 3,6-epoxy-2-hydroxymethyl-6-methyl-7-octenoic acid[11] (**7**, 20.0 mg), respectively.

The BuOH-soluble hydrolysate (500 mg each, 3 times) was separated by MPLC (column: Lichroprep RP_{18}, 40−63 μm, size B; solvent: MeOH−H_2O, 6:4; flow rate: 2.0 mL/min) to give five fractions (fraction 1, 600 mg; fraction 2, 440 mg; fraction 3, 53 mg; fraction 4, 240 mg; fraction 5, 89 mg). Parts of fractions 1 (100 mg) and 2 (100 mg) were subjected to preparative TLC with $CHCl_3$−MeOH−H_2O (7:3:0.5 and 8:4:0.5, respectively) to give (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside (**8**, 56.4 mg) and prosapogenins **9** (26.3 mg) and **13**[5,14] (9.7 mg), respectively. In turn, a part of fraction 4 (150 mg) was subjected to MPLC (column: Lichroprep Si60, 40−63 μm, size B; solvent: $CHCl_3$−MeOH−H_2O, 8:4:0.5; flow rate: 2.0 mL/min) to yield four fractions (fraction 4−1, 11 mg; fraction 4−2, 73 mg; fraction 4−3, 53 mg; fraction 4−4, 4 mg). Subfractions 2 and 3 were separated by preparative TLC with $CHCl_3$−MeOH−H_2O−HOAc (8:4:0.5:0.01) to give prosapogenins **10** (18.7 mg) and **11**[12] (13.9 mg) and prosapogenin **12**[13] (24.7 mg), respectively.

Another part (50 mg) of the BuOH-soluble hydrolysate was treated with 5 M H_2SO_4−1,4-dioxane (1:1, 10 mL) at 90 °C for 12 h. The reaction mixture was extracted with EtOAc (10 mL × 3), and the extract was concentrated in vacuo and purified by preparative TLC with MeOH−$CHCl_3$ (1:30) to give acacic acid lactone[5] (**14**, 2.5 mg).

(2*E*)-6-Hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-Quinovopyranoside (8): light yellow oil; $[\alpha]^{25}_D$ +17.2° (*c* 0.10, MeOH); ^{1}H (CD_3OD, 400 MHz) and ^{13}C (CD_3OD, 100 MHz) NMR, see Table 3; FABMS *m/z* 369.2 $[M + Na]^+$, 347.2 $[M + H]^+$, 329.2 $[M + H - H_2O]^+$; HRFABMS *m/z* 347.1723 (calcd for $C_{16}H_{27}O_8$ $[M + H]^+$, 347.1706).

Prosapogenin 9: colorless amorphous solid; $[\alpha]^{25}_D$ +1.1° (*c* 0.53, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) aglycon δ 5.30 (1H, br s, H-12), 4.48 (1H, br s, H-16), 4.12 (1H, dd, J = 11.4, 5.3 Hz, H-21), 3.05 (1H, dd, J = 14.0, 4.0 Hz, H-18), 1.37 (3H, s, H_3-27), 1.08 (3H, s, H_3-29), 0.95 (3H, s, H_3-25), 0.93 (6H, s, H_3-23, H_3-30), 0.85 (3H, s, H_3-24), 0.79 (3H, s, H_3-26); for other NMR data, see Tables 1 and 2; FABMS *m/z* 967.5 $[M + Na]^+$, 795.3 $[M + H - H_2O - ara]^+$, 765.3 $[M + H - H_2O - glc]^+$, 471.1 $[M + H - H_2O - glc - ara - glc]^+$; HRFABMS *m/z* 967.4852 (calcd for $C_{47}H_{76}O_{19}Na$ $[M + Na]^+$, 967.4879).

Prosapogenin 10: colorless amorphous solid; $[\alpha]^{25}_D$ −35.7° (*c* 0.47, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) aglycon δ 5.30 (1H, br s, H-12), 4.48 (1H, br s, H-16), 4.13 (1H, dd, J = 11.2, 5.4 Hz, H-21), 3.11 (1H, dd, J = 11.5, 4.2 Hz, H-3), 3.03 (1H, dd, J = 13.7, 4.0 Hz, H-18), 1.37 (3H, s, H_3-27), 0.95 (3H, s, H_3-29), 0.94 (3H, s, H_3-25), 0.93 (6H, s, H_3-23, H_3-30), 0.78 (3H, s, H_3-26), 0.76 (3H, s, H_3-24); for other NMR data, see Tables 1 and 2; FABMS *m/z* 846.5 $[M + Na]^+$, 824.5 $[M + H]^+$, 806.5 $[M + H - H_2O]^+$; HRFABMS *m/z* 846.4616 (calcd for $C_{43}H_{69}O_{14}NNa$ $[M + Na]^+$, 846.4616), 824.4798 (calcd for $C_{43}H_{70}O_{14}N$ $[M + H]^+$, 824.4796).

Methylation and Enzymatic Hydrolysis of Monoterpenoid 8. Monoterpenoid **8** (20.0 mg) was methylated with diazomethane, followed by purification with preparative TLC (MeOH−$CHCl_3$, 7:3), to give methyl (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoate 6-*O*-β-D-quinovopyranoside[6] (10.5 mg). Enzymatic hydrolysis of the methyl ester with β-glucosidase from almonds (30 mg; Sigma, 2.4 units/mg) in acetate buffer (1 mL, pH 5.0) for 2 days, followed by Si gel (3 g) column chromatography with MeOH−$CHCl_3$ (9:1), gave methyl (2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoate[6] (3.1 mg).

Cytotoxicity Assay. Cytotoxicity against human HT-1080 fibrosarcoma cells was determined using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide assay as described previously.[22] The ED_{50} value of each compound was: **1**, 4.89 μg/mL (2.83 μM); **2**, 1.43 μg/mL (0.70 μM); **3**, 1.87 μg/mL (0.91 μM); **4**−**13**, > 100 μg/mL; 5-fluorouracil, 1.05 μg/mL (8.0 μM).

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Supporting Information Available: Figure 2, showing significant HMBC correlations for prosapogenins **9** and **10** and FABMS fragment ions for prosapogenin **9**, and Figure 3, showing significant HMBC correlations for kinmoonoside A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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