Two Major Saponins from Seeds of Barringtonia asiatica : Putative Antifeedants toward Epilachna sp. Larvae

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Two Major Saponins from Seeds of *Barringtonia asiatica*: Putative Antifeedants toward *Epilachna* sp. Larvae

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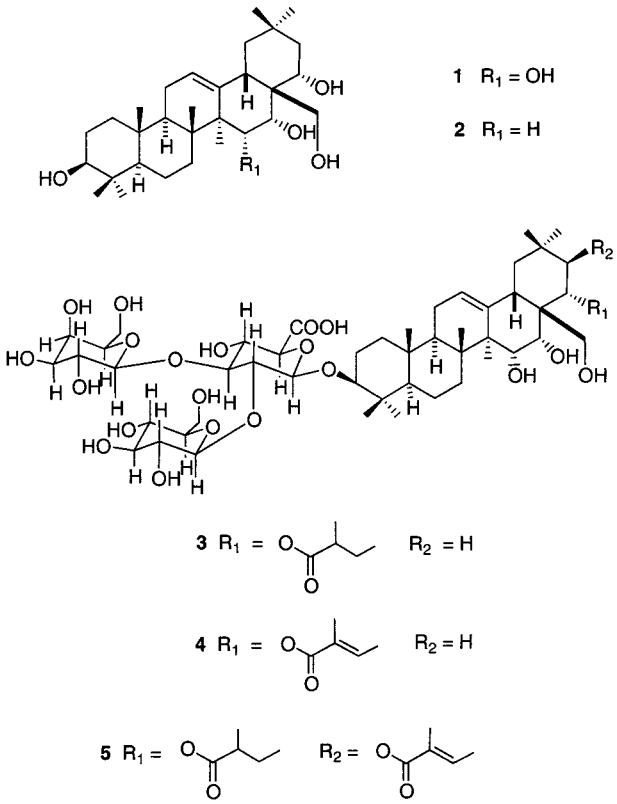
Two major saponins have been isolated from a methanol extract of the seeds of *Barringtonia asiatica*, and their structures elucidated (mainly by two-dimensional NMR spectroscopy) as 3-*O*-{[*β*-D-galactopyranosyl(1→3)-*β*-D-glucopyranosyl(1→2)]-*β*-D-glucuronopyranosyloxy}-22-*O*-(2-methylbutyroyloxy)-15,16,28-trihydroxy-(3*β*,15α,16α,22α)-olean-12-ene (**3**) and 3-*O*-{[*β*-D-galactopyranosyl(1→3)-*β*-D-glucopyranosyl(1→2)]-*β*-D-glucuronopyranosyloxy}-22-*O*-[2(*E*)-methyl-2-butenyloyloxy]-15,16,28-trihydroxy-(3*β*,15α,16α,22α)-olean-12-ene (**4**). The antifeedant properties of **3** and **4** toward *Epilachna* larvae are discussed.

Barringtonia asiatica Kurz (Lecythidacaeae), also known as the "fish killer tree", grows extensively in coastal regions of tropical Asia and the Pacific, including northern Australia. The seeds when ground up and thrown into water exhibit piscicidal activity, which has been exploited by native fisherman in these regions for centuries to enhance their harvest. It is believed that the piscicidal activity arises from damage to the gill capillaries of the fish, and the latter, unable to absorb dissolved oxygen in the water via their gills, rise to the surface to breathe through their mouths.[1] Recent research has shown that a methanol extract of the seeds of *B. asiatica*, when coated on leaves of various *Solanum* species, is also a powerful antifeedant toward larvae of *Epilachna* species.[2] These larvae constitute a considerable agricultural threat to Indonesia's pistachio nut and potato crops, and the use of specific and ecologically friendly control measures therefore has considerable advantages over conventional pesticides.

There has been surprisingly little published work on the isolation of natural products from *B. asiatica*. Nozoe,[3] in 1934, extracted a mixture of saponins (A_1-barrinin) from *B. asiatica*. On acidic hydrolysis, A_1-barrinin produced a mixture of sapogenins (A_1-barrigenin), together with a mixture of several sugars that were later shown[4] to be fucose, galactose, glucose, and glucuronic acid in a ratio of 1:2:2:2. On basic hydrolysis, A_1-barrigenin gave two aglycons (A_1-barrigenol and A_2-barrigenol) together with tiglic acid. In 1967 the structure of A_1-barrigenol was deduced as 3*β*,15α,16α,22α,28*β*-pentahydroxyolean-12-ene (**1**) from ^{1}H NMR spectroscopy.[5] A_2-barrigenol was found by the same authors[5] to be identical to camelliagenin A, whose structure had been deduced previously as 3*β*,16α,22α,28*β*-tetrahydroxyolean-12-ene[6] (**2**), i.e., 15-deoxy-A_1-barrigenol.



Results and Discussion

In the present study, methanol extractives of the seeds were partitioned between 1-butanol and water.[2] Purification of the 1-butanol extractives by silica gel chromatography produced a fraction that was homogeneous by TLC in various solvent systems and exhibited strong antifeedant activity toward *Epilachna* species. However, analytical reversed-phase HPLC using UV detection showed two major peaks that eluted well after the void volume and constituted about one-quarter of the mass of the 1-butanol extractives, followed by several more minor peaks.

The major, second-eluting compound (**3**) in the 1-butanol extractives was purified by semipreparative HPLC using a methanol−tetrahydrofuran−acetic acid−water mobile phase (see Experimental Section). Negative FABMS gave a quasi molecular ion peak at *m/z* 1073 and indicated a molecular weight of 1074. The quasi molecular ion peak showed two successive losses of 162 mass units from the $[M - 1]^-$ ion. Following treatment of **3** with anhydrous methanolic HCl and per-trimethylsilylation, the GC profile

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Table 1. NMR Data (in CD_3OD) for *B. asiatica* Saponins

carbon	δC (ppm) **3**	δC (ppm) **4**	δC (ppm)[a]	δC (ppm)[b]	δH (ppm), multiplicity, *J* (Hz) **3**	δH (ppm), multiplicity, *J* (Hz) **4**
aglycon						
1	38.9	39.6			1.38, dm, 12.5; 2.07, dm, 12.5	1.36, dm, 12.0; 2.06, dm, 12.0
2	27.2	26.7			1.80, dm, 12.0; 2.15, dm, 12.0	1.79, dm, 11.5; 2.10, dm, 11.5
3	89.4	89.3			3.30, dd, 3.5, 11.0	3.28, dd, 4.0, 11.5
4	39.6	38.9				
5	55.5	55.4			0.80	0.79
6	18.8	18.8			1.39, br.d, 12.5; 1.60, br.d, 12.5	1.36, m;
7	36.7	36.7			2.13, m	2.06, m; 2.17, m
8	41.5	41.7				
9	47.1	47.1				
10	36.7	36.7				
11	23.9	24.0			1.74, dm, 11.0; 1.87, dm, 11.0	1.75, dm, 11.0; 1.87, dm, 11.0
12	124.8	124.8			5.46, t, 3.2	5.45, t, 3.2
13	144.5	144.5				
14	47.8	47.8				
15	67.5	67.6			4.24, d, 5.5	4.24, m
16	74.7	74.7			4.49, m	4.57, m
17	45.2	45.5				
18	41.6	41.6				
19	47.0	47.0			2.87, dd, 12.5, 14.0; 3.03, dd, 4.0, 14.0	2.87, dd, 13.0, 14.0; 3.02, dd, 3.5, 14.0
20	31.9	32.0				
21	41.6	41.5			1.95, dd, 6.0, 11.0; 2.78, dd, 11.5, 12.0	2.06, dd, 5.5, 11.0; 2.80, dd, 11.0, 12.0
22	72.0	71.9			6.15, dd, 6, 12	6.15, dd, 5.5, 12.0
23	27.9	27.9			1.24, s	1.22, s
24	16.7	16.8			1.10, s	1.09, s
25	15.8	15.8			0.83, s	0.81, s
26	17.5	17.6			1.03, s	1.01, s
27	21.3	21.4			1.87, s	1.87, s
28	62.8	62.9			3.59, d, 10.5; 3.78, d, 10.5	3.59, d, 10.5; 3.75, d, 10.5
29	33.5	33.6			1.07, s	1.05, s
30	25.2	25.3			1.26, s	1.27, s
glucuronic acid						
1′	105.2	105.3	105.2	103.2	4.93, d, 7.5	4.94, d, 7.5
2′	78.8	79.1	79.0	76.7	4.43, m	4.43, m
3′	87.5	87.8	88.0	83.9	4.36, m	4.42, m
4′	71.7	71.9	71.9	68.6	4.51, m	4.51, m
5′	77.2	77.3	76.4	76.2	4.55, m	4.55, m
6′	170.6 (Me ester)	172.0	171.9	174.4		
glucose						
1″	103.8	104.0	103.9	101.2	5.66, d, 8.0	5.65, d, 7.0
2″	76.3	76.4	75.4	74.3	4.09, dd, 8.0, 9.0	4.08, dd, 7.0, 8.0
3″	78.4	78.6	78.6	76.3	4.23, m	4.24, m
4″	72.4	72.5	70.9	70.5	4.15, m	4.15, m
5″	77.7	77.4	77.6	75.4	3.81, ddd, 4.0, 5.0, 5.0	3.81, m
6″	63.1	63.4	63.6	61.5	4.33, m; 4.45, m	4.37, m; 4.46, m
galactose						
1‴	105.2	105.3	105.2	102.5	5.31, d, 7.5	5.30, d, 7.5
2‴	72.9	73.0	73.0	71.1	4.48, m	4.50, m
3‴	75.3	75.4	72.8	73.0	4.14, m	4.14, m
4‴	70.0	70.2	70.2	70.6	4.46, m	4.46, m
5‴	77.2	77.4	77.4	74.3	4.16, m	4.15, m
6‴	61.9	62.0	62.1	60.8	4.33, m; 4.45, m	4.35, m; 4.45, m
ester						
1⁗	176.2	167.9				
2⁗	41.8	129.8			2.29, dq, 7.0	
3⁗	26.7	136.4			1.36, ddt, 6.0, 7.0, 15.0; 1.68, ddt, 6.0, 7.0, 15.0	6.82, q, 7.0
4⁗	11.8	14.1			0.84, s	1.42, d, 7.0
5⁗	16.7	12.3			1.10, d, 7.0	1.76, s

[a] Data from ref 9. [b] Data from ref 10.

of the products was compared with reference sugars treated under the same conditions. This indicated that D-galactose, D-glucose, and D-glucuronic acid were present in the ratio 1:1:1. The 162 mass unit losses observed in the negative FABMS could thus be interpreted as being due to sequential fragmentation of one galactose and one glucose unit from the parent saponin. The D-configuration has been assumed for these sugars in keeping with Massiot and Lavaud's assertion regarding the D-sugars commonly found in saponins:[7] "The enantiomers of these sugars are not found in plants, a fact used as a clue in the determination of these sugars". The IR spectrum of **3** showed a strong absorption at 1709 cm^{-1}, indicating the presence of an ester group, and a weaker absorption at 1638 cm^{-1}, indicating the presence of a carbon−carbon double bond.

The 500 MHz ^1H NMR spectrum in d_5-pyridine of **3** (Table 1) showed seven methyl groups on quaternary carbons and an olefinic proton at 5.46 ppm, while methylene protons at 3.59 and 3.78 ppm indicated a hydroxymethyl substituent. Three anomeric sugar protons were observed at 4.93, 5.31, and 5.66 ppm, with their coupling constants (Table 1) indicating that the sugars were β-linked

both to the aglycon and to one another. The 125 MHz ^{13}C NMR spectrum (Table 1) was consistent with an oxygen-substituted olean-12-ene skeleton, with further structural information being provided by 2-D NMR techniques (COSY, HMBC, and HMQC-TOCSY; see Table 2 for correlations).

Elucidation of the connectivity commenced with the anomeric sugar proton at 4.94 ppm (H-1′) that in HMBC showed a long-range correlation to a carbon at 89.4 ppm, which was directly connected, from HMQC, to a methine proton as a doublet of doublets at 3.30 ppm. Comparison with published data for an olean-12-ene saponin[8] indicated that the carbon at 89.4 ppm very likely belonged to C-3 of the aglycon, and thus the methine proton at 3.30 ppm was designated as H-3. From COSY, H-3 showed coupling to a methylene group at 1.80/2.15 ppm (H-2), and this in turn showed coupling to another methylene group at 1.38/2.07 ppm (H-1). C-3 by HMBC showed long-range correlations to two methyl groups at 1.24 (H-23) and 1.10 (H-24), and both of these showed long-range correlation to carbons at 39.6 ppm (C-4) and 55.4 ppm (C-5). From HMQC, C-5 was connected to a proton at 0.80 ppm (H-5), which in turn showed correlations to two more methylene groups at 1.39/1.60 ppm (H-6) and 2.13 ppm (H-7). The carbon attached to the latter proton, 36.7 ppm (C-7), showed a long-range correlation by HMBC to a methyl group at 0.83 ppm (H-25), which in turn showed long-range correlations to carbons at 41.5 (C-8), 47.1 (C-9), and 36.7 ppm (C-10). C-9 by HMBC showed a long-range correlation to the olefinic proton at 5.46 ppm (H-12), and the latter from COSY was coupled to a methylene group at 1.74/1.87 ppm (H-11). H-12 from HMQC was directly connected to a carbon at 124.8 ppm (C-12), while H-11 from HMBC showed a long-range correlation to a quaternary carbon at 144.5 ppm (C-13). H-12 from HMBC showed a long-range correlation to a carbon at 47.8 ppm (C-14), and the latter, also from HMBC, showed long-range correlations to two methyl groups at 1.03 ppm (H-26) and 1.87 ppm (H-27), as well as to a methine doublet at 4.24 ppm (H-15). The latter, from HMBC, showed a long-range correlation to the carbon at 21.3 ppm (C-27) that was directly connected to H-27 from HMQC. From COSY, H-15 was coupled directly to a methine multiplet at 4.47 ppm (H-16), and the latter, from HMBC, showed a long-range correlation to a quaternary carbon at 45.2 ppm (C-17). The low-field shifts of H-15 and H-16 indicated they were oxygen bearing, and the low-field shift of the methyl at 1.87 ppm (H-27) is characteristic of 16α-hydroxyoleananes. The olefinic proton at 5.46 ppm (H-12) by HMBC also showed a long-range correlation to a carbon at 41.6 ppm (C-18), which in turn, from HMQC-TOCSY, showed a long-range correlation to a methylene group at 2.87/3.03 ppm (H-19). H-19 from HMBC showed long-range correlation to a quaternary carbon at 31.9 ppm (C-20), which in turn showed long-range correlations to two more methyl groups at 1.07 ppm (H-29) and 1.26 ppm (H-30), and to another methylene group at 1.95/2.78 ppm (H-21). H-21 from COSY was coupled to a very low-field methine proton at 6.15 ppm (H-22). This methine proton at 6.15 ppm was connected directly, from HMQC, to a carbon at 72.0 ppm (C-22), and the latter showed a long-range correlation from HMBC to a sharp doublet at 1.10 ppm (H-5′′′′). This doublet showed correlations from HMBC to a sharp triplet at 0.84 ppm (H-4′′′′), two resonances at 1.36 and 1.68 ppm (H-3′′′′) that each appeared as a doublet of doublet of triplets, and to a doublet of quartets at 2.29 ppm (H-2′′′′). These signals were attributed to a 2-methylbutyrate moiety attached to C-22 of the aglycon. H-22 from HMBC also showed long-range correlation to a carbon at 62.8 ppm (C-28), and the latter was directly connected to the oxygen-bearing methylene group at 3.59/3.78 ppm (H-28). C-28 from HMBC also showed long-range correlations to the two low-field protons at 4.49 ppm (H-16) and 4.24 (H-15) ppm, which completed the circumnavigation of the sapogenin. This NMR analysis enabled us to assign the A_1-barrigenol structure (**1**) to the parent aglycon, which is substituted at C-22 with 2-methylbutyrate. This sapogenin has been previously isolated and characterized by Higuchi et al. from *Pittosporum undulatum*.[9]

Because of overlap of some hydrogen and carbon resonances, the sugar structure of this saponin proved particularly challenging to elucidate. Moreover, the carboxyl carbon of the glucuronic acid unit was not observed in the ^{13}C NMR spectrum. However, treatment of **3** with excess diazomethane afforded a methyl ester and the carboxyl carbon was now readily observable at 170.6 ppm. HMQC-TOCSY established that the anomeric proton of the sugar connected directly to the aglycon (4.93 ppm) (H-1′) was also connected to a carbon at 105.2 (C-1′) ppm. The carbons adjacent to this carbon were then shown by this technique to be (in sequence) at 78.8 (C-2′), 87.5 (C-3′), 71.7 (C-4′), and 77.2 (C-5′) ppm. Since HMQC-TOCSY showed only five hydrogen-bearing carbons in this six-carbon moiety, the sugar directly connected to the aglycon had to be D-glucuronic acid. This is in keeping with the structural data for many saponins that contain a uronic acid moiety.[1]

C-2′ of the D-glucuronic acid moiety showed a long-range correlation by HMBC to another sugar anomeric proton at 5.66 ppm (H-1′′). HMQC-TOCSY showed the latter was connected to the carbon at 103.8 ppm (C-1′′) and then established that the next five carbons in sequence in this sugar were at 76.3 (C-2′′), 78.4 (C-3′′), 72.4 (C-4′′), 77.7 (C-5′′), and 63.1 (C-6′′) ppm. C-3′ of the D-glucuronic acid moiety showed a long-range correlation by HMBC to the third anomeric proton at 5.31 (H-1′′′) ppm. This anomeric proton was similarly shown by HMQC-TOCSY to be connected to a carbon at 105.2 (C-1′′′) ppm and that the next five carbons in this sugar were established to be at 72.9 (C-2′′′), 75.3 (C-3′′′), 70.0 (C-4′′′), 77.2 (C-5′′′), and 61.9 (C-6′′′) ppm.

It only remained to elucidate whether D-glucose was connected to C-2′ and D-galactose to C-3′ of the D-glucuronic acid moiety, or vice versa. The carbon shifts of the three sugars gave an excellent match with literature data[9] (see Table 1) for D-glucose connected to C-2′ and D-galactose connected to C-3′ of D-glucuronic acid, whereas a poor match was obtained with data[10] from saponins in which this connectivity was interchanged (Table 1). The complete saponin structure of **3** was therefore deduced as 3-*O*-{[*β*-D-galactopyranosyl(1→3)-*β*-D-glucopyranosyl(1→2)]-*β*-D-glucuronopyranosyloxy}-22-*O*-(2-methylbutyroyloxy)-15,16,28-trihydroxy-(3*β*,15α,16α,22α)-olean-12-ene. This compound has already been obtained by Higuchi et al.[11] from *P. undulatum*, but only as a mixture with the C-22 3,3-dimethylacryloyl ester.

The first eluting and less abundant of the two major saponins (**4**) gave a quasi molecular ion by negative FABMS at *m*/*z* 1071, which indicated a molecular weight of 1072, but lacked the sequential 162 mass unit fragmentations observed in the more abundant saponin (**3**). Since the molecular weight of **4** was 2 mass units lower than **3**, it was anticipated to have one more degree of unsaturation. Hydrolysis and derivatization of the sugars produced an identical GC profile to that from **3**, thus confirming that both saponins contained the same saccharide substituents. The IR spectrum showed absorptions at 1685 and 1645

Table 2. NMR Correlations Observed in *B. asiatica* Saponins (One-Bond Correlations Shown in Bold)

carbon	^{1}H−^{1}H COSY **3**	^{1}H−^{1}H COSY **4**	^{1}H−^{13}C HMQC-TOCSY **3**	^{1}H−^{13}C HMQC-TOCSY **4**	^{1}H−^{13}C HMBC **3**	^{1}H−^{13}C HMBC **4**
1	H-1, H-2	H-1, H-2				H-3
2	H-1, H-2, H-3	H-1, H-2, H-3			H-24, H-25	H-3
3	H-2	H-2	H-1, H-2, **H-3**, H-25	H-1, H-2, **H-3**, H-25	H-1′, H-24, H-25	H-23, H-24
4					H-23, H-24	H-23, H-24
5		H-6	**H-5**, H-6, H-25	**H-5**, H-6, H-25	H-23, H-24, H-25	H-23, H-24, H-25
6	H-7	H-5, H-7	**H-6**, H-7, H-25	H-6		
7	H-6	H-6	**H-7**, H-25	H-6, **H-7**, H-25		
8			H-25, H-27		H-11, H-12, H-15, H-26	
9					H-12, H-25	
10					H-25	H-25, H-26
11	H-11, H-12	H-11, H-12				H-12
12	H-11	H-11	**H-12**, H-19	H-11, **H-12**		H-19, H-27
13					H-11	H-12, H-19, H-27
14			H-18, H-19		H-12, H-15, H-26, H-27	H-12, H-15, H-26, H-27
15	H-16	H-16	**H-15**, H-16	**H-15**, H-16		H-16, H-27
16	H-15	H-15	H-15, **H-16**	H-15, **H-16**	H-22	H-22
17					H-16, H-22	H-16, H-19, H-22
18	H-19	H-19		**H-18**, H-19	H-12	H-12,
19	H-18, H-19	H-18, H-19	H-18, **H-19**	H-18, **H-19**	H-16, H-29, H-30	H-29, H-30
20					H-19, H-21, H-29, H-30	H-21, H29, H-30
21	H-21, H-22	H-21, H-22	**H-21**, H-22, H-30	**H-21**, H-22	H-16, H-22, H-29, H-30	H-22, H-29, H-30
22	H-21	H-21	H-21, **H-22**	H-21, **H-22**	H-15, H-21, H-28, H-5′′′′	H-21, H-28
23			**H-23**	H-23	H-24	H-24
24			**H-24**	H-24	H-23	H-5, H-23
25			**H-25**, H-27	**H-25**		
26			**H-26**	**H-26**		
27			**H-27**	**H-27**	H-15	H-15
28	H-28	H-28	**H-28**	**H-28**	H-22	H-15, H-22
29			**H-29**	**H-29**	H-11, H-21, H-30	H-30
30			**H-30**	**H-30**	H-29	H-29
1′	H-2′	H-2′	**H-1′**, H-2′, H-3′, H-4′, H-5′	**H-1′**, H-2′, H-3′, H-4′, H-5′	H-4′	H-3, H-3′, H-4′, H-5′
2′	H-1′	H-1′, H-3′	H-1′, **H-2′**, H-3′, H-4′, H-5′	H-1′, **H-2′**, H-3′, H-4′, H-5′	H-1′′	H-1′′
3′		H-2′	H-1′, H-2′, **H-3′**, H-4′, H-5′	H-1′, H-2′, **H-3′**, H-4′, H-5′	H-1′′′	H-1′′′, H-2′, H-4′, H-5′
4′		H-5′	H-1′, H-3′, **H-4′**, H-5′	H-1′, H-2′, H-3′, **H-4′**, H-5′		
5′		H-4′	H-1′, H-2′, H-4′, **H-5′**	H-1′, H-2′, H-3′, H-4′, **H-5′**		H-2′, H-3′
6′						H-4′, H-5′
1′′	H-2′′	H-2′′	**H-1′′**, H-2′′, H-3′′, H-4′′, H-5′′	**H-1′′**, H-2′′, H-3′′, H-4′′, H-5′′	H-2′, H-4′′	H-2′, H-2′′
2′′	H-1′′	H-1′′, H-3′′	H-1′′, **H-2′′**, H-3′′, H-4′′, H-5′′	H-1′′, **H-2′′**, H-3′′, H-4′′, H-5′′		
3′′		H-2′′, H-4′′	H-1′′, H-2′′, **H-3′′**, H-4′′, H-5′′	H-1′′, H-2′′, **H-3′′**, H-4′′, H-5′′		H-6′′
4′′		H-3′′, H-5′′	H-1′′, H-2′′, H-3′′, **H-4′′**, H-5′′, H-6′′	H-1′′, H-2′′, H-3′′, **H-4′′**, H-5′′		H-6′′
5′′		H-4′′, H-6′′	H-1′′, H-2′′, H-3′′, H-4′′,**H-5′′**	H-1′′, H-2′′, H-3′′, H-4′′, **H-5′′**, H-6′′		
6′′		H-5′′	H-3′′, H-4′′, H-5′′, **H-6′′**	H-2′′, H-3′′, H-4′′, H-5′′, **H-6′′**		H-4′′
1′′′	H-2′′′	H-2′′′	**H-1′′′**, H-2′′′, H-3′′′, H-4′′′, H-5′′′	**H-1′′′**, H-2′′′, H-3′′′, H-4′′′, H-5′′′	H-3′, H-4′′	H-3′, H-2′′′, H-3′′′, H-4′′′
2′′′	H-1′′′	H-1′′′, H-3′′′	H-1′′′, **H-2′′′**, H-3′′′	H-1′′′, **H-2′′′**, H-3′′′, H-4′′′		
3′′′		H-2′′′, H-4′′′	H-1′′′, H-2′′′, **H-3′′′**	H-1′′′, H-2′′′, H-3′′′, H-4′′′		H-4′′′, H-6′′′
4′′′	H-5′′′	H-3′′′	H-1′′′, H-2′′′, H-3′′′, **H-4′′′**, H-5′′′	H-1′′′, H-2′′′, H-3′′′, **H-4′′′**, H-5′′′		H-3′′′, H-5′′′, H-6′′′
5′′′	H-4′′′, H-6′′′	H-4′′′, H-6′′′	**H-5′′′**, H-6′′′	H-1′′′, H-2′′′, H-4′′′, **H-5′′′**, H-6′′′		
6′′′	H-5′′′	H-5′′′	H-5′′′, **H-6′′′**	H-5′′′, **H-6′′′**		H-3′′′, H-5′′′
1′′′′					H-22, H-2′′′′, H-3′′′′, H-5′′′′	H-22, H-3′′′′, H-4′′′′, H-5′′′′
2′′′′	H-5′′′′		H-5′′′′		H-3′′′′, H-4′′′′, H-5′′′′	H-3′′′′, H-4′′′′, H-5′′′′
3′′′′	H-4′′′′	H-4′′′′, H-5′′′′	H-2′′′′, H-4′′′′, H-5′′′′	**H-3′′′′**, H-4′′′′, H-5′′′′	H-1′′′′, H-2′′′′, H-4′′′′	H-4′′′′, H-5′′′′
4′′′′	H-3′′′′	H-3′′′′, H-5′′′′	H-3′′′′, **H-4′′′′**, H-5′′′′	H-3′′′′, **H-4′′′′**, H-5′′′′	H-2′′′′, H-3′′′′	H-3′′′′
5′′′′	H-2′′′′	H-3′′′′, H-4′′′′	H-2′′′′, H-4′′′′, **H-5′′′′**	H-4′′′′, **H-5′′′′**	H-2′′′′, H-3′′′′, H-4′′′′	H-3′′′′

Table 3. Antifeedant Activity of *B. asiatica* Saponins Toward *Epilachna* sp.

saponin	conc (μg mL^{-1})	activity (%)
3	1000	100.00
	500	54.54
	100	0.00
	50	0.00
4	1000	100.00
	500	63.35
	100	39.09
	50	10.00

cm^{-1} indicative of an unsaturated ester, and basic hydrolysis[3] subsequently afforded tiglic acid (as had been observed by Nozoe[3]), identified by GC−MS of the methyl ester. Comparison of the ^{1}H and ^{13}C NMR shift and connectivity data with that of **3** (Tables 1 and 2) indicated that **4** possessed the same aglycon and sugar sequence as **3**. However in **4**, H-22 from HMBC showed a long-range correlation to a carboxyl carbon at 167.9 ppm (C-1′′′′), with the latter showing long-range correlations to an olefinic proton at 6.82 ppm (H-3′′′′). H-3′′′′ appeared as a quartet (J = 7.0 Hz) and from COSY was coupled to a methyl doublet (J = 7.0 Hz) at 1.42 ppm (C-4′′′′) and another methyl singlet at 1.76 ppm (C-5′′′′). The low-field shift of H-3′′′′ indicated that these two methyl groups were *cis* to one another and, suppported by the degradation evidence (see above), established that in this saponin the aglycon was esterified at C-22 with the tiglate moiety. The complete saponin structure of **4** is thus 3-*O*-{[β-D-galactopyranosyl-(1→3)-β-D-glucopyranosyl(1→2)]-β-D-glucuronopyranosyloxy}-22-*O*-[2(*E*)-methyl-2-butenyloyloxy]-15,16,28-trihydroxy-(3β,15α,16α,22α)-olean-12-ene. Although the sapogenin of **4** may have been obtained in the degradation of saponins isolated from *Eryngium planum*,[12] this structure was not conclusively assigned. To the best of our knowledge **4** is a new compound, since an exhaustive substructure search using SciFinder failed to find any references to it.

The antifeedant properties of **3** and **4** were investigated by coating methanol solutions of these saponins at concentrations of 1000, 500, 100, and 50 μg mL^{-1} on to half of a *Solanum niger* leaf, the other half (the control) being coated only with methanol. The antifeedant properties of the saponins were determined by observing how much of the saponin-coated leaf relative to the control half of the leaf was consumed by *Epilachna* larvae. These data are presented in Table 3 and indicate that the saponin containing tiglate (**4**) had considerably higher antifeedant activity than the saponin containing 2-methylbutyrate (**3**).

After completing the structural analysis of these two saponins we noted an Internet reference[13] to recent work carried out by Burton[14] that was not included in *Dissertation Abstracts*. Burton describes the isolation and structural elucidation of a major saponin from *B. asiatica* seeds collected in Western Samoa, which showed the highest activity in a brine shrimp mortality assay of all the HPLC fractions obtained from this source. This saponin (**5**) has the same sugar sequence as the two saponins we have isolated, but instead incorporates the R_1-barrigenol aglycon in which C-21 is also oxygen-bearing and in this case is esterified with tiglate. The chromatogram of the 1-butanol extractives of our *B. asiatica* seeds when run under the same chromatographic conditions as described by Burton showed considerable differences from the chromatogram obtained by him. It appears that in our 1-butanol extractives Burton's saponin (**5**) was either absent or was present only in very small amounts. We did see a very small peak in the electrospray MS, which had the same molecular weight (1172) as **5**. It is likely that the saponin content of *B. asiatica* seeds varies with geographical location, an observation also made by Burton.[15]

A portion of the 1-butanol extractives were also degraded to the free aglycons emulating Nozoe's conditions,[3] whereby A_1-barrigenol (**1**) and cammelliagenin A (**2**) were isolated, thus confirming Nozoe's observations. Unlike earlier reports,[4] no D-fucose was observed in the GC profile of the 1-butanol extractives after methanolysis and per-trimethylsilylation, an outcome that may suggest a further variation of saponin content of *B. asiatica* seeds with geographical location.

Preliminary work on the minor saponins of *B. asiatica* indicates that several are derived from camelliagenin A (**2**), which also supports Nozoe's observations.[3] They also appear to have less antifeedant activity toward *Epilachna* than those derived from A_1-barrigenol (**1**). Derivation of these structures and their antifeedant properties will be discussed in a future publication.

Experimental Section

General Experimental Procedures. Semipreparative HPLC was performed on YMC-Pack ODS-AQ 5 μm 120 Å 250 mm columns of 10 and 20 mm internal diameter, thermostated at 40 °C. The mobile phase was generated by blending (A) methanol−tetrahydrofuran−water−acetic acid, 9:1:90:0.05, and (B) methanol−tetrahydrofuran−acetic acid, 90:10:0.05, in the proportions 60% B/40% A. Flow rates were 4 mL min^{-1} (10 mm i.d. column) and 10 mL min^{-1} (20 mm i.d. column). Instrumentation for HPLC consisted of two Waters 510 pumps, a Rheodyne 7125 injector fitted with a 4.4 mL sample loop, and a Waters 481 UV/visible detector fitted with a 2.3 mm path flow cell, monitoring absorbance at 210 nm. The instrumentation was interfaced with Waters Maxima software, and fractions were collected manually. Optical rotation values were measured in a 1 dm path cell with a Perkin-Elmer 241 polarimeter. IR spectra as 1% dispersions in KBr were obtained on a Perkin-Elmer 1800 FTIR spectrophotometer. NMR spectra of the saponins dissolved in d_5-pyridine were acquired on a Varian Inova instrument at 500 MHz (proton) and 125 MHz (carbon) in 5 mm tubes. The 2-D NMR pulse sequences were provided by Varian. Negative FABMS were measured in a 3-nitrobenzyl alcohol matrix on a VG Analytical ZAB SEQ2 mass spectrometer.

Plant Material. Seeds from *B. asiatica* were collected from Sanghi Talaud, North Sulawesi Province, Indonesia, in January 1996 and identified by Mr Djuandi. A specimen, voucher number 3033, is kept in the Biology Department, Technology Institute of Bandung, Bandung, Indonesia.

Bioassay Procedure. Saponins **3** and **4** were dissolved in methanol to give the following concentrations: 50, 100, 500, and 1000 μg mL^{-1}. These solutions were applied to the left half of the leaves of *Solanum nigrum*, while methanol only as control was applied to the right half of the leaves. The leaves were then dried and placed in Petri dishes that contained wet tissue and case cloth and covered with a small dish containing a 3.5 cm hole in the center. The larvae from instar stages 3−4 that had been deprived of food for 2 h were introduced singly into the Petri dishes and were allowed to remain in the Petri dishes for 24 h before removal. The amount of leaf consumed was calculated by dividing the leaf into 32 sectors. Antifeedant activities were calculated from the formula $[(C - T)/(C + T)] \times 100$, where C and T, respectively, represent the amount of control and treatment portions of the leaves consumed by larvae.

Extraction and Isolation. Following removal of the outer seed covering, 1 kg of the kernels was crushed and extracted repeatedly with methanol to give 20 g of a dark brown, resinous material. This material was partitioned between equal volumes of 1-butanol and water. Diethyl ether was then added to the 1-butanol extractives, precipitating about 1 g of

a buff-colored powder. This crude saponin-containing material was dissolved in methanol, diluted with 40% water, and a volume equivalent to 100 mg injected onto the 20 mm i.d. column that was eluted with 60% B mobile phase (see above for HPLC conditions). Following manual collection of the saponin fractions, the column was washed with 100% B mobile phase before re-equilibration with 60% B and repeat injection of the crude saponin-containing material. The saponin-containing fractions from the first HPLC purification were rotary evaporated to dryness and redissolved in 60% B mobile phase, and a volume equivalent to 20 mg was injected onto the 10 mm i.d. column that was eluted with 60% B mobile phase. The purified saponin fractions from each injection of the second HPLC purification were pooled, concentrated as before, and dried under high vacuum for 1 day to remove acetic acid.

Saponin (3): white amorphous solid, 170 mg; $[\alpha]^{22}_D$ +0.7° (3.06 mg mL^{-1} CH_3OH); IR (KBr) ν_{max} 3427, 2945, 1709, 1638, 1464, 1387, 1261, 1202, 1157, 1076, 1041, 1019 cm^{-1}; NMR (see Table 1); FABMS (see text); high-resolution negative FABMS *m*/*z* 1073.5619 [M − 1]$^+$ (calcd for $C_{53}H_{85}O_{22}$, 1073.5508); *anal.* C 55.6%, H 7.7%; calcd for $C_{53}H_{86}O_{22} + CH_3COOH + 3H_2O$, C 55.5%, H 8.1%.

Saponin (4): white amorphous solid, 70 mg; $[\alpha]^{22}_D$ +4.6° (2.63 mg mL^{-1} CH_3OH); IR (KBr) ν_{max} 3429, 2947, 1685, 1648, 1521, 1387, 1278, 1157, 1076, 1041 cm^{-1}; NMR (see Table 1); FABMS (see text); high-resolution negative FABMS *m*/*z* 1071.5429 [M − 1]$^+$ (calcd for $C_{53}H_{83}O_{22}$, 1071.5352); *anal.* C 55.5%, H 7.7%; calcd for $C_{53}H_{84}O_{22} + CH_3COOH + 3H_2O$, C 55.6%, H 8.0%.

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