Bioactive Triterpene Saponins from the Roots of *Phytolacca americana*

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Five new triterpene saponins named phytolaccasaponins N-1 (**1**), N-2 (**2**), N-3 (**3**) N-4 (**4**), and N-5 (**5**) were isolated from the roots of *Phytolacca americana* together with seven known triterpene saponins (**6**−**12**). The structures of the five new saponins were established as shown in structures **1**−**5** on the basis of their spectroscopic data. The MDR-reversal activity of **1**−**12** was evaluated on the basis of the amount of calcein accumulated in MDR human ovarian cancer 2780 AD cells in the presence of each compound. The most effective compound was **8** (155% of control at 25 μg/mL).

Phytolacca esculenta Van Houtt. (syn.: *Phytolacca acinosa* Roxb.) (Phytolaccaceae) and *Phytolacca americana* L. (Phytolaccaceae) are native to the south of mainland China and the eastern United States, respectively. Their dried roots are used as a traditional herbal medicine and a folk medicine in China and are called "Shang-Lu" and "Chui-Xu Shang-Lu", respectively, which are used for the treatment of tumors, edema, bronchitis, and abscesses.[1a,b] *Phytolacca americana* is a toxic plant in the United States and causes vomiting when the fruits, young leaves, and roots are consumed.[1c] Both plants were introduced to Japan from their native countries, with *P. americana* now found there in both grasslands and open forests. The constituents of *P. esculenta*[1–4,8–11,15] and *P. americana*[1,6,7,12–14] have been investigated by many researchers with an interest in their biological activities. We have conducted a further investigation on the roots of *P. americana* and describe the saponins obtained and their biological activities.

Results and Discussion

A methanol extract of air-dried roots of *P. americana* was partitioned successively with hexane, ethyl acetate, and *n*-BuOH. From the *n*-BuOH-soluble portion, five new triterpene saponins, phytolaccasaponins N-1 (**1**), N-2 (**2**), N-3 (**3**) N-4 (**4**), and N-5 (**5**), were isolated, together with seven known triterpene saponins (**6**−**12**).

Phytolaccasaponin N-1 (**1**) gave the elemental composition $C_{48}H_{76}O_{22}$, as determined by HRFABMS analysis. The IR spectrum indicated the presence of hydroxy (3436 cm^{-1}) and ester carbonyl (1724 cm^{-1}) groups. The ^{13}C NMR spectrum displayed 48 carbon resonances (Table 1), with two carbonyl carbons resonating at δ 176.8 and 176.1 and two olefin carbon resonances located at δ 145.2 (qC) and 123.3 (CH). Four resonances for carbons bearing oxygen were observed at δ 82.9 (CH), 71.2 (CH), 66.9 (CH), and 65.4 (CH_2) in addition to one methoxy and 17 oxygenated carbon resonances of two hexose sugars and one pentose sugar. From the DEPT and HMQC spectra, the remaining carbon resonances were for five methyls, eight methylenes, three methines, and six quaternary carbons. The ^1H NMR spectrum showed five methyl signals (δ 1.82, 1.38, 1.34, 1.26, and 1.09) (Table 2). The connectivity of the protonated carbons was determined from the $^1H-^1H$ COSY spectrum (Table S1). A HMBC experiment was used to determine the carbon−carbon connection through the nonprotonated carbon atoms (HMBC correlations: CH_3-24 to C-5 and C-4; CH_3-25 to C-1 and C-5; CH_3-26 to C-7, C-8, and C-14; CH_3-27 to

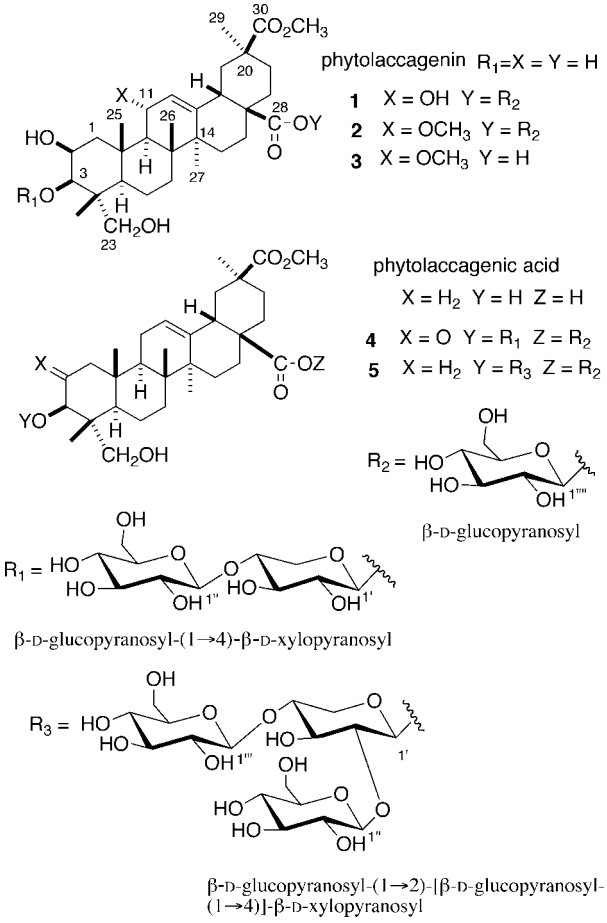

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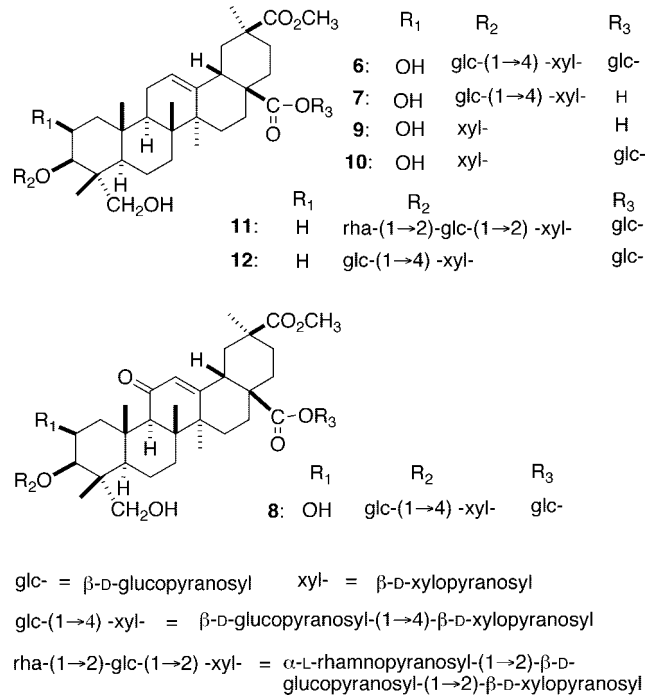
Table 1. ^{13}C NMR Spectroscopic Data (125 MHz,C_5D_5N) for Compounds **1**–**5**[a]

	1	2	3	4	5
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	46.4, CH_2	45.0, CH_2	45.0, CH_2	54.6, CH_2	38.7, CH_2
2	71.2, CH	71.1, CH	71.1, CH	208.5, qC	26.1, CH_2
3	82.9, CH	82.7, CH	82.7, CH	83.7, CH	82.0, CH
4	43.0, qC	42.9, qC	43.0, qC	43.3, qC	43.5, qC
5	48.0, CH	47.8, CH	47.7, CH	47.1, CH	48.0, CH
6	18.2, CH_2	18.1, CH_2	18.0, CH_2	18.5, CH_2	18.3, CH_2
7	33.7, CH_2	33.3, CH_2	33.4, CH_2	32.4, CH_2	32.9, CH_2
8	43.6, qC	42.3, qC	43.1, qC	40.2, qC	39.9, qC
9	56.9, CH	53.9, CH	53.8, CH	47.7, CH	48.2, CH
10	38.5, qC	38.3, qC	38.3, qC	38.3, qC	36.9, qC
11	66.9, CH	76.2, CH	76.2, CH	23.7, CH_2	23.5, CH_2
12	123.3, CH	123.0, CH	122.8, CH	123.0, CH	123.6, CH
13	145.2, qC	148.2, qC	148.9, qC	143.8, qC	143.8, qC
14	42.4, qC	43.4, qC	42.3, qC	42.1, qC	42.0, qC
15	28.3, CH_2	28.3, CH_2	28.5, CH_2	28.3, CH_2	28.3, CH_2
16	23.5, CH_2	23.4, CH_2	23.7, CH_2	23.4, CH_2	23.8, CH_2
17	46.3, qC	46.1, qC	45.7, qC	46.4, qC	46.5, qC
18	42.5, CH	42.6, CH	42.8, CH	43.1, CH	43.2, CH
19	41.8, CH_2	42.2, CH_2	42.5, CH_2	42.3, CH_2	42.4, CH_2
20	43.9, qC	43.9, qC	44.1, qC	43.9, qC	44.0, qC
21	30.5, CH_2	30.5, CH_2	30.7, CH_2	30.5, CH_2	30.6, CH_2
22	33.9, CH_2	33.9, CH_2	34.5, CH_2	33.9, CH_2	34.0, CH_2
23	65.4, CH_2	65.1, CH_2	65.2, CH_2	63.7, CH_2	64.7, CH_2
24	15.0, CH_3	14.8, CH_3	14.8, CH_3	13.9, CH_3	13.4, CH_3
25	18.9, CH_3	19.0, CH_3	19.0, CH_3	16.7, CH_3	16.1, CH_3
26	19.2, CH_3	19.3, CH_3	19.2, CH_3	17.1, CH_3	17.5, CH_3
27	25.9, CH_3	25.4, CH_3	25.4, CH_3	26.0, CH_3	26.1, CH_3
28	176.1, qC	176.1, qC	180.0, qC	176.0, qC	176.0, qC
29	28.2, CH_3	28.2, CH_3	28.3, CH_3	28.3, CH_3	28.3, CH_3
30	176.8, qC	176.8, qC	177.0, qC	176.9, qC	176.9, qC
OMe	51.6, CH_3	51.7, CH_3	51.7, CH_3	51.7, CH_3	51.7, CH_3
OMe-11		54.3, CH_3	54.3, CH_3		
xyl-1′	106.4, CH	106.4, CH	106.4, CH	105.7, CH	104.4, CH
2′	75.1, CH	75.0, CH	75.0, CH	74.5, CH	83.1, CH
3′	76.4, CH	76.4, CH	76.3, CH	75.8, CH	75.9, CH
4′	77.3, CH	77.4, CH	77.4, CH	77.6, CH	77.4, CH
5′	64.7, CH_2	64.7, CH_2	64.7, CH_2	64.7, CH_2	64.2, CH_2
glc-1″	103.5, CH	103.5, CH	103.5, CH	103.5, CH	105.9, CH
2″	74.1, CH	74.1, CH	74.1, CH	74.1, CH	76.8, CH
3″	78.8, CH	78.9, CH	78.1, CH	78.9, CH	78.9, CH
4″	71.7, CH	71.7, CH	71.6, CH	71.7, CH	71.5, CH
5″	78.9, CH	78.9, CH	78.9, CH	78.9, CH	78.2, CH
6″	62.6, CH_2	62.6, CH_2	62.6, CH_2	62.6, CH_2	62.6, CH_2
glc-1‴					103.5, CH
2‴					74.4, CH
3‴					78.8, CH
4″					71.7, CH
5‴					78.3, CH
6‴					62.7, CH_2
glc-1⁗	95.8, CH	95.8, CH		95.8, CH	95.8, CH
2⁗	74.2, CH	74.1, CH		74.1, CH	74.2, CH
3⁗	79.4, CH	79.4, CH		79.4, CH	79.3, CH
4⁗	70.9, CH	70.9, CH		70.9, CH	71.0, CH
5⁗	78.1, CH	78.1, CH		78.1, CH	78.0, CH
6⁗	61.9, CH_2	61.9, CH_2		61.8, CH_2	62.0, CH_2

[a] Signals were assigned from $^1H-^1H$ COSY, HMQC, and HMBC spectra.

C-8, C-13, and C-14; CH_3-29 to C-20, C-21, and C-30; H-12 to C-14 and C-18; H-1⁗ to C-28). Interpretation of these results suggested that compound **1** is a triterpene saponin possessing an oleanane skeleton bearing five methyl groups at C-4, C-8, C-10, C-14, and C-20 and a methoxycarbonyl group at C-20.

The HMBC correlations [H-3 (δ 4.29, 1H) to C-2 (δ 71.2, CH); H-1′ (δ 4.95, 1H) to C-3 (δ 82.9, CH)] were used to place a hydroxy group and an *O*-glycosyl bond at C-2 and C-3, respectively. The HMBC correlation [H-9 (δ 2.06, 1H) to C-11 (δ 66.9, CH)] and the $^1H-^1H$ COSY correlation [H-11 (δ 4.70, 1H) with H-9 and H-12 (δ 5.90, 1H)] were used to situate a hydroxy group at C-11 and a trisubstituted carbon−carbon double bond between C-12 and



C-13. The HMBC correlation of CH_3-24 to the signal due to a hydroxymethyl carbon at δ 65.4 (CH_2) was used to place a hydroxymethyl group at C-4. The NOESY experiment was used for assignment of the relative configuration of the oleanane skeleton **1**. Several NOESY correlations [H-2 with H-3; H-3 with H-1α, H-1′, H-2, H-5, and H_2-23; H-9 with H-1α and CH_3-27; H-18 with H-12 and H-19β; CH_3-25 with H-1β, H-11, CH_3-24, and CH_3-26; CH_3-29 with H_2-21; H-5⁗ (the sugar proton of glucosyloxycarbonyl at C-17) with OCH_3 (the methoxycarbonyl at C-20)] were used to determine the full stereostructure of the aglycone of phytolaccasaponin N-1, 11α-hydroxyphytolaccagenin.

The sugar portions of **1** were assigned as 3-*O*-β-glucopyranosyl-(1→4)-β-xylopyranoside and 28-*O*-β-glucopyranoside on the basis of comparisons of the ^{13}C and ^1H NMR data with those published for analogous compounds. These assignments were supported by NOESY correlations [(H-1″ with H-4′; H-2″ with H-4″); (H-1′ with H-3′ and H-3); (C-28 glucosyl: H-1⁗ with H-5⁗ and H-3⁗; H-5⁗ with H-1⁗ and OCH_3)], as well as coupling constants observed [H-1″ (d, J = 7.8 Hz); H-1′ (d, J = 7.6 Hz), H-2′ (dd, J = 7.6, 8.4 Hz); C-28 glucosyl: H-1⁗ (d, J = 8.1 Hz)]. The C-4′−C-1″ linkage of xylose and glucose was determined by the analysis of HMBC (H-1″ to C-4′) and NOESY (between H-4′ and H-1″) correlations (Table S1). Since only D-glucose and D-xylose are known in *P. esculenta* [2,3,8–11,15] and *P. americana*,[6,7,12–14] the sugar portions in **1** were assigned as 3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-xylopyranoside] and 28-*O*-(β-D-glucopyranoside). In the course of isolation of **1**, esclentoside H (**6**)[8] was isolated as the most abundant product. The structure of **6** had already been determined unambiguously by acid hydrolysis.[8] The δ_C values of compound **1** were in good agreement with those of **6** including around the glycosyl bonds at C-3 and C-28 and sugar moieties[16] (Table 1 and Table S6) except those of the carbons that were influenced by the hydroxy group at C-11 of **1**. In addition, the $[\alpha]_D$ values of **1** (+11.1) and **6** (+44.1)[8] had the same sign. Accordingly, compound **1** was assigned as 28-*O*-(β-D-glucopyranosyl)-3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-11α-hydroxyphytolaccagenin.

Phytolaccasaponin N-2 (**2**) gave the elemental composition $C_{49}H_{78}O_{22}$, as determined by HRFABMS. The IR spectrum indicated the presence of hydroxy (3480 cm^{-1}) and ester carbonyl (1742 cm^{-1}) groups, and the ^{13}C NMR spectrum displayed 49 carbon resonances. The ^1H and ^{13}C NMR data of **2** were in general accordance with those of **1** except in the chemical shifts for C-11

Table 2. ^{1}H NMR Data (500 MHz, C_5D_5N) for Compounds **1**–**5**[a]

	1	**2**	**3**	**4**	**5**	
position	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	
1β	3.31, dd (2.8, 14.9)	2.63, dd (3.0, 14.5)	2.65, dd (2.2, 14.7)	2.19, d (12.0)	1.55, m	
1α	1.96, m	1.74, m	1.76, m	2.43, d (11.7)	0.98, m	
2	4.76, m	4.71, m	4.72, m		2β 1.34, m; 2α 1.13, m	
3	4.29, m		4.24, d (3.7)	5.09, s	4.08, m	
5	1.89, m	1.88, m	1.86, br d (11.5)	1.79, br d (12.0)	1.56, m	
6	1.57, m	1.54, m	1.56, m	1.64, m	1.55, m	
7β	1.32, m	1.54, m	1.56, m	1.64, m	1.28, m	
7α		1.30, m	1.23, m	1.31, m		
9	2.06, m	1.97, m	2.00, m	2.02, m	1.98, m	
11	4.70, m	3.98, m	3.99, m	11β 1.90, m; 11α 1.75, m	1.90, m	
12	5.90, d (3.9)	5.82, d (3.7)	5.86, d (3.9)	5.50, dd (3.2, 3.2)	5.55, t (3.9)	
15	2.06, m	2.04, m	2.03, m	2.02, m	2.06, m	
16	1.89, m	2.02, m	2.06, m	2.04, m	1.97, m	
18	3.24, dd (3.9, 13.4)	3.30, dd (3.7, 13.4)	3.38, dd (3.4, 13.5)	3.19, dd (3.9, 13.8)	3.20, dd (3.9, 12.9)	
19β	2.20, m	2.3, m	2.30, m	2.19, m	2.20, m	
19α	1.67, m	1.74, m	1.76, m	1.75, m	1.74, m	
21β	2.06, m	2.04, m	2.16, d (12.9)	2.02, m	2.06, m	
21α		1.41, m	1.45, m		1.34, m	
22β	1.89, m	1.88, m	2.06, m	1.90, m	1.90, m	
22α			2.00, m			
23	4.37, m	4.36, m	4.36, d (10.5)	4.36, m	4.27, m	
	3.69, d (10.0)	3.66, d (10.7)	3.67, d (10.5)	3.71, d (10.7)	3.70, d (10.5)	
24	1.38, s	1.35, s	1.34, s	0.84, s	1.03, s	
25	1.82, s	1.71, s	1.68, s	0.94, s	0.95, s	
26	1.26, s	1.17, s	1.06, s	1.05, s	1.10, s	
27	1.34, s	1.28, s	1.33, s	1.20, s	1.18, s	
29	1.09, s	1.13, s	1.16, s	1.16, s	1.16, s	
OMe	3.53, s	3.58, s	3.63, s	3.58, s	3.58, s	
OMe-11		3.20, s	3.23, s			
xyl-1′	4.95, d (7.6)	4.95, d (7.6)	4.96, d (7.6)	4.98, d (7.4)	1′ 4.92, d (6.8)	
2′	3.91, dd (7.6, 8.4)	3.91, dd (7.6, 8.4)	3.91, dd (7.6, 8.3)	4.09, dd (7.4, 7.4)	2′ 4.07, m	
3′	4.05, m	4.05, m	4.07, m	4.11, m	3′ 4.10, m	
4′	4.18, m	4.23, m	4.19, m	4.17, m	4′ 4.20, m	
5′β	4.29, m	4.33, m	4.33, m	4.41, dd (5.4, 11.7)	5′β 4.29, m	
5′α	3.53, m	3.56, dd (8.2, 10.3)	3.57, m	3.61, m	5′α 3.52, dd (9.2, 11.5)	
glc-1″	5.02, d, 7.8	5.02, d (7.8)	5.03, d (7.8)	4.99, d (8.1)	1″ 5.29, d (7.6)	
2″	4.05, m	4.05, m	4.04, dd (7.8, 7.8)	4.01, t (8.1)	2″ 4.02, m	
3″	4.29, m	4.25, m	4.20, d, (7.8)	4.24, m	3″ 3.89, m	
4″	4.18, m	4.18, m	4.12, m	4.36, m	4″ 4.20, m	
5″	4.29, m	4.23, m	3.99, m	3.96, m	5″ 3.89, m	
6″a	4.54, dd (2.0, 11.7)	4.38, m	4.55, dd (2.2, 11.7)	4.52, dd (2.0, 11.5)	6″a 4.47, dd (2.5, 11.7)	
6″b	3.97, m	3.98, m	4.32, dd (5.6, 11.7)	4.29, dd (5.8, 11.7)	6″b 4.33, m	
glc-1‴	6.31, d (8.1)	6.32, d (8.1)		6.28, d (8.1)	glc-1‴ 4.95, d (7.8)	glc-1‴ 6.24, d (8.1)
2‴	4.18, m	4.18, m		4.17, m	2‴ 3.95, m	2‴ 4.10, m
3‴	3.96, m	3.98, m		3.96, m	3‴ 4.20, m	3‴ 3.95, m
4‴	4.38, m	4.38, m		4.17, m	4‴ 4.18, m	4‴ 4.30, m
5‴	4.18, m	4.33, m		4.24, m	5‴ 3.89, m	5‴ 4.13, m
6‴a	4.37, m	4.55, dd (2.2, 11.5)		4.36, m	6‴a 4.37, dd (3.9, 11.5)	6‴a 4.30, m
6‴b	3.97, m	4.33, m			6‴b 4.44, dd (5.2, 11.7)	6‴b 4.27, m

[a] Signal were assingned from ^{1}H−^{1}H COSY, HMQC, and HMBC spectra.

[^{13}C NMR (δ 76.2, CH) and ^{1}H NMR (δ 3.98, 1H, m)] and the observation of an additional methyl group present [^{13}C NMR (δ 54.3, CH_3) and ^{1}H NMR (δ 3.20, 3H, s)]. HMBC correlations [H-11(δ 3.98, m) to OCH_3-11 (δ 54.3); OCH_3-11 (δ 3.20, s) to C-11 (δ 76.2, CH)] were used to place the methoxy group in **2** at C-11. NOESY correlations (H-11 with H-12, CH_3-25, CH_3-26, and OCH_3-11; OCH_3-11 with H-11 and H-12) were employed to confirm the α-orientation of this OMe-11 group. Thus, the structure of phytolaccasaponin N-2 (**2**) was deduced as 28-*O*-(β-D-glucopyranosyl)-3-*O*-[β-D-glucopyranosyl(1→4)-β-D-xylopyranosyl]-11α-methoxyphytolaccagenin.[16] This was supported by the full analysis of its ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY NMR correlations (Table S2).

Phytolaccasaponin N-3 (**3**) gave the elemental composition $C_{43}H_{68}O_{17}$ from the HRFABMS. The IR (KBr) spectrum indicated the presence of hydroxy (3440 cm^{-1}) and ester carbonyl (1734 cm^{-1}) groups and a trisubstituted carbon−carbon double bond (1638 cm^{-1}), with the ^{13}C NMR spectrum displaying 43 carbon resonances. The ^{1}H and ^{13}C NMR data of **3** were in good accordance with those of **2** except for the chemical shift of C-28 in their ^{13}C NMR spectra [^{13}C NMR (δ 180.0, qC)] and the disappearance of ^{1}H and ^{13}C NMR signals for a 28-*O*-glucosyl unit in compound **3** (Tables 1, 2, and S3). Phytolaccoside E[13] (esculentoside A)[15] (**7**) was isolated as a major product in the course of the isolation of **3**, and the structure of **7** had already been determined. The only difference in their structures is the substituent at C-11. The δ_C values of compounds **3** were in good agreement with those of **7** including the resonances around the glycosyl bonds at C-3 and C-28 and sugar moieties[16] (Table S6), except those of the carbons that were influenced by the methoxy group at C-11 of **3**. In addition, $[\alpha]_D$ values of **3** (+62.6) and **7** (+45.0)[15] had the same sign. Thus, the full structure of phytolaccasaponin N-3 (**3**) was proposed as 3-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl]-11α-methoxyphytolaccagenin.

Phytolaccasaponin N-4 (**4**) gave the elemental composition $C_{48}H_{74}O_{21}$ by HRFABMS. The ^{13}C NMR spectrum displayed 48 carbon resonances, with the data of C-12 through C-22, C-28, C-29, C-30, and the one pentose and two hexose units (C-1′ through C-5′; C-1″ through C-6″; C-1‴ through C-6‴) in **4** being in good accordance with those of **1** and **2**. Thus, the D and E rings of a phytolaccagenin-related unit and the connectivity of the sugar portions 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-xylopyranoside and 28-*O*-β-D-glucopyranoside in **4** were observed to be the same as those of **1** and **2**. The chemical shift at C-11 (δ 23.7, CH_2) of **4** in the ^{13}C NMR spectrum indicated that the hydroxyl group [C-11 (δ 66.9, CH)] of **1** and the methoxy group [C-11 (δ 76.2, CH)] of **2** were displaced by the methylene hydrogen in **4**. The chemical shift

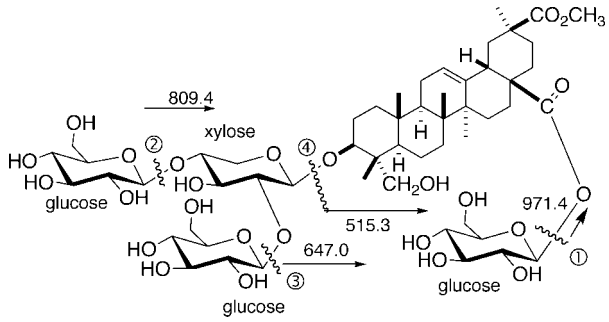


Figure 1. ESI negative mass fragmentation (*m*/*z*) values of **5** determined by MS/MS experiment.

of C-2 (δ 208.5, qC) of **4** indicated that the hydroxyl groups of **1**, **2**, and **3** at C-2 are displaced by a carbonyl oxygen in **4**.

The connectivity of the protonated carbons was determined from the ^{1}H−^{1}H COSY spectrum of **4**. A HMBC experiment was used to confirm the carbon−carbon connections through nonprotonated carbon atoms (Table S4). Interpretation of these results suggested that compound **4** has a phytolaccagenic acid structure with a carbonyl group at C-2. The glucosyloxycarbonyl group was placed at C-17, as in **1** and **2**, because of the good accordance of the chemical shifts of C-16, C-17, C-22, and C-28 of **4** with those of corresponding carbons of **1** and **2**. The NOESY correlation (Table S4) indicated the full stereostructure of phytolaccasaponin N-4 as shown in structure **4**. Thus, compound **4** was assigned as 28-*O*-(β-D-glucopyranosyl)-3-*O*-[β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl]-2-oxophytolaccagenic acid.

Phytolaccasaponin N-5 (**5**) gave the elemental composition $C_{54}H_{86}O_{25}$ by HRESIMS. The ^{13}C NMR spectrum displayed 54 carbon resonances, with signals for C-8, C-9, C-11 through C-22, C-26, C-27, C-28, C-29, C-30, and OMe being superimposable with those of **4** (Table 1). The ^{13}C NMR data of the glucosyloxy carbons (C-1′′′′ through C-6′′′′) at C-28 of **5** were also superimposable with those of **1**, **2**, and **4**. Thus, the upper half of structure **5** (C, D, and E rings) could be designated the same as that of **4** including the relative configuration (C-8, C-9, C-14, C-17, C-18, and C-20). The carbonyl carbon (δ 208.5, qC) of **4** at C-2 was displaced by a methylene carbon (δ 26.1, CH_2) in **5**. The structures of the A and B rings of **5** were confirmed by the analyses of its NMR spectra. The connectivity of the protonated carbons was determined by the ^{1}H−^{1}H COSY spectrum. A HMBC experiment was used to confirm the carbon−carbon connections through nonprotonated carbons. Several NOESY correlations (H-3 with H-1′, H-5, and CH_2-23; H-5 with H-9 and CH_2-23; CH_3-24 with CH_3-25) indicated the full structure of the A and B rings of **5**, as shown (Tables S5-1).

The structure of the glycosyl portions of the 3-*O*-glycosyl and 17-glycosyloxycarbonyl units of **5** were determined by the analysis of ESIMS and ^{13}C and ^{1}H NMR data. Thus, ESIMS analysis of **5** based on MS/MS experiment (Figure 1) was used to place a glucopyranosyloxy carbonyl bond at C-17 and a glucopyranosyl-(1→2)[glucopyranosyl-(1→4)]-xylopyranosyl bond at the C-3β position. HMBC correlations [H-1′ to C-3 (δ 82.0, CH_2); H-1′′′′ to C-28 (δ 176.0, qC)] and a NOESY correlation (between H-1′ and H-3) were supportive of these conclusions. In addition, HMBC correlations (H-1′′ to C-2′; H-1′′′ to C-4′) and NOESY correlations (between H-1′′ and H-2′; between H-1′′′ and H-4′) were used to place two β-glucopyranosyloxy bonds at the C-2′ and C-4′ positions of the β-xylopyranosyl moiety (Table S5-2). Since only D-glucose and D-xylose are known in *P. esculenta*[2,3,8–11,15] and *P. americana*,[6,7,12–14] the glycosyl moiety at the C-3 position was deduced to be 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-xylopyranoside. Therefore, compound **5** was assigned as 28-*O*-(β-D-glucopyranosyl)-3-*O*-{β-D-glucopyranosyl-(1→2)[β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl}phytolaccagenic acid (**5**).

A further seven structurally related triterpene saponins were also isolated in the present investigation: esculentoside H (**6**),[2,8] esculentoside A (phytolacoside E) (**7**),[2,12,13,15] esculentoside M (**8**),[10] esculentoside B (phytolaccoside B) (**9**),[12,13,15] esculentoside S (**10**),[2,15] 28-*O*-(β-D-glucopyranosyl) esculentoside R (**11**),[2] esculentoside L (**12**).[11] The identification of compounds **6**−**12** was carried out by determination of the physical and spectroscopic parameters and by comparison of their ^{13}C NMR spectra with those of the corresponding compounds reported in the literature (Tables S6 and S7).

One of the mechanisms of multidrug resistance (MDR) by cancer cells is overexpression of P-glycoprotein (P-gp) and the transport of anticancer agents from the inside to the outside of cancer cells.[17a,b] In the present study, effects of saponins **1**−**12** as MDR reversal agents were estimated by the increase of cellular accumulation of the fluorogenic dye calcein. When the effects of saponins **1**−**12** on the cellular accumulation of calcein in MDR human ovarian cancer 2780AD cells were examined, [18a,b] all compounds except **11** showed weak to significant effects on the accumulation of calcein in MDR 2780AD cells by comparison with a control (Table 3). It is interesting to note that while the cytotoxic activity of compound **8** toward WI-38, VA-13, and HepG2 cells was very weak (IC_{50} >97 μM/mL), it exhibited a significant effect on the accumulation of calcein (155% of control at 25 μg/mL) in MDR 2789AD, because cytotoxicity is unnecessary for MDR cancer-reversal agents. Thus, compound **8** is a potential lead compound as a MDR cancer-reversal agent.

The cytotoxic activities of **1**−**12** were evaluated against three cell lines, namely, WI-38, VA-13, and HepG2 cells.[18a,b,20] The assay results indicated that no compound showed significant cytotoxic activity toward VA-13 malignant tumor cells and HepG2 human cancer cells.

The potential anti-inflammatory activity of the isolated compounds **1**−**12** was estimated *in vitro* by inhibition of the induction of ICAM-1 in the presence of IL-1α using human cultured cell line A549 cells, an *in vitro* human endothelial cell model.[19a,b,20,21a,b] The data for **1**−**12** indicated that they have no activity toward inhibition of the induction of ICAM-1.

Experimental Section

General Experimental Procedures. Melting points are uncorrected and obtained using a Yanagimoto micromelting point instrument. Optical rotations were measured using a Horiba Sepa-200 polarimeter. IR spectra were recorded on a Hitachi 270-30 infrared spectrometer. ^{1}H and ^{13}C NMR spectra were measured with a Varian Unity-plus instrument at 500 and 125 MHz. ^{1}H NMR assignments were determined by ^{1}H−^{1}H COSY experiments, and ^{13}C NMR assignments determined using DEPT, HMQC, and HMBC experiments. HRFABMS was recorded on a JEOL JMS-HX110 instrument, and HRESIMS was recorded on a JEOL JMS-700TZ instrument. HPLC separations were performed on a Hitachi L-6200 HPLC instrument with an Inertsil Prep-sil GL 10 × 250 mm stainless steel column and monitored by a Hitachi L-7400 UV detector and a Shodex SE-61 RI detector. To describe chromatographic separation, the column and solvent are designated in this order. Column codes are as follows: A, column chromatography (CC) on silica gel (70–200 mesh); B, flash chromatography (FC) on silica gel (230–400 mesh); C, Inertsil-ODS, 25 × 1 cm i.d. stainless column. Solvent codes are as follows: a, MeOH−MeCN−H_2O (3:3:4); b, $CHCl_3$−MeOH−H_2O (6:4:0.5); c, MeOH−MeCN−H_2O (1:1:3); d, $CHCl_3$−MeOH−H_2O.

Plant Material. The roots of *Phytolacca americana* were collected in Niigata City, Niigata Province, Japan, in October 2000. The plant was identified by Dr. K. Yonekura, Deparment of Biology, Faculty of Science, Tohoku University, Sendai, Japan. A voucher specimen was deposited at the Deparment of Chemistry and Chemical Engineering, Niigata University.

Extraction and Isolation. The air-dried roots (6.23 kg) were extracted with MeOH (30 L × 2) for 7 days. The MeOH extract was concentrated to 3.0 L, and water (500 mL) was added. This was extracted with hexane (5 × 1.1 L), EtOAc (4.5 L), and *n*-butanol

Table 3. Effect of Compounds on the Accumulation of Calcein in Mutidrug-Resistant 2780 AD Cells, Cytotoxic Activities of Compounds against WI-38, VA-13, and HepG2 Cells, and Effect of Compounds on Induction of ICAM-1 and on Cell Viability

	calcein accumlation (% of control)[a,b]			cytotoxicity IC_{50} (μM)[c]				
compound	0.25 μg/mL	2.5 μg/mL	25 μg/mL	WI-38	VA-13	HepG2	inhibitory activity of induction of ICAM-1 (μM)[d]	cell viability by MTT assay (μM)[e]
1	121	119	124	>100	94.0	>100	>100	>100
2	105	100	108	>98.2	>98.2	>98.2	>98.2	>98.2
3	118	123	135	>118	>118	>118	>118	>118
4	98	115	120	>101	>101	>101	>101	>101
5	126	106	123	>88.2	>88.2	>88.2	>88.2	>88.2
6	124	129	123	>101	>101	>101	>320	>1012
7	111	104	119	7.1	117	102	>383	661
8	119	135	155	>98.8	>98.8	97.4	>98.8	>98.8
9	128	117	123	36.2	68.9	74.7	253	265
10	141	132	143	>121	95.2	>121	>121	>121
11	93	68	94	>89.0	>89.0	>89.0	>89.0	>89.0
12	124	101	126	>103	>103	>103	>103	>103

[a] The amount of calcein accumulated in multidrug-resistant human ovarian cancer 2780AD cells was determined with the control in the presence of 0.25, 2.5, and 25 μg/mL of compounds. [b] The values are the relative amount of calcein accumulated in the cell compared with control. [c] IC_{50} represents the mean of duplicate determination. [d] A549 cells were preptreated with various concentrations of the compounds for 1 h and then incubated in the presence of IL-1α for 6 h. Absorbance of 415 nm was assayed after treatment of the cell with primary and secondary antibodies and addition of the enzyme substrate as described in the Experimental Section. The experiments were carried out in triplicate cultures. [e] A549 cells were incubated with serial dilutions of the compounds for 24 h. Cell viability (%) was measured by MTT assay and used for determination of IC_{50}. Experiments were carried out in triplicate cultures.

(4.0 L), successively. The *n*-butanol extract (75.1 g) was separated by CC [A, d (gradient)] into F1−F11.

Fraction F5 (22.1 g) was separated into F5-1−F5-6 by CC [A, d (gradient)]. F5-2 (3.10 g) was separated by HPLC (C, a) to give **7** [1.18 g (0.01895%)]. F5-3 (3.97 g) was subjected to FC [B, d (7:3:0.5)] to give F5-3-1−F5-3-3, and separation of F5-3-3 in the same conditions gave F5-3-3-1−F5-3-3-4. Separation of F5-3-3-2 (1.60 g) by HPLC [C, a] afforded F5-3-3-2-3 (103.1 mg), which was purified by HPLC [C, a] to give **3** [39.4 mg (0.00063%)] and **10** [87.1 mg (0.00140%)]. F5-5 (6.23 g) was subjected to FC [B, d (8:2:0.3)] to give F5-5-2 (5.90 g). This subfraction was separated by HPLC [C, a] to give **2** [72.8 mg, (0.00117%)], **4** [64.1 mg (0.00103%)], and **6** [4.89 g (0.07852%)].

Fraction 6 (17.40 g) was separated into F6-1−F6-6 by CC [A, d (gradient)]. F6-2 (6.85 g) was subjected to FC (B, b) to give **6** (3.97 g, 0.0638%). F6-3 (7.58 g) was subjected to FC (B, b) to give F6-3-1−F6-3-5. F6-3-2 gave five fractions by FC (B, b). F6-3-2-2 (1.38 g) was purified by HPLC (C, c) to give **1** [17.0 mg, (0.00027%)], **2** [15.2 mg (0.00024%)], and **8** [22.8 mg (0.00037%)].

All inseparable subfractions of F6 were combined and named F6′. F6′ (13.0 g) was separated into F6-1′−F6-8′ by FC (B, b). F6-3′ (290 mg) was separated by HPLC [C, MeOH−MeCN–H_2O (3:3:5)] to give **12** [21.8 mg (0.00035%)]. F6-5′ (6.63 g) was separated into F6-5-1′−F6-5-8′ by FC (B, b). F6-5-5′ was separated into five fractions by FC (B, b). F6-5-5-4′ (189 mg) was purified by HPLC (C, c) to give **5** [28.5 mg (0.00046%)]. Then, F6-5-5-3′ (1.67 g) was separated by HPLC [C, c] into F6-5-5-3-1′−F6-5-5-3-10′. F6-5-5-3-10′ (85.8 mg) was purified by HPLC [C, c] to give **11** [54.8 mg (0.00088%)].

The EtOAc extracts (56.30 g) was separated by CC [A, d (gradient)] into F1−F5. Fraction F2 (6.0 g) was separated into F2-1−F2-3 by FC [B, d (gradient)]. Fraction F2-2 eluted by [d (68:15:1.7)] gave a viscous oil (2.56 g), which was purified by repeated HPLC [C, a] to give **9** [611 mg (0.00981%)].

Phytolaccasaponin N-1 (1): white powder; mp 214–216 °C; $[\alpha]^{20}_D$ +11.1 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3436, 1724 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and S1; HRFABMS (positive) *m/z* 1027.4710 (calcd for $C_{48}H_{76}O_{22}Na$, 1027.4727).

Phytolaccasaponin N-2 (2): white powder; mp 194–195 °C; $[\alpha]^{20}_D$ +18.9 (*c* 0.71, MeOH); IR (KBr) ν_{max} 3480, 1742 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and S2; HRFABMS (positive) *m/z* 1041.4870 (calcd for $C_{49}H_{78}O_{22}Na$, 1041.4882).

Phytolaccasaponin N-3 (3): white powder; mp 213–215 °C; $[\alpha]^{20}_D$ +62.6 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3440, 1734 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and S3; HRFABMS (positive) *m/z* 879.4363 (calcd for $C_{43}H_{68}O_{17}Na$, 879.4355).

Phytolaccasaponin N-4 (4): white powder; mp 205–207 °C; $[\alpha]^{20}_D$ +26.0 (*c* 0.84, MeOH); IR (KBr) ν_{max} 3416, 1726 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and S4; HRFABMS (positive) *m/z* 1009.4630 (calcd for $C_{48}H_{74}O_{21}Na$, 1009.4621).

Phytolaccasaponin N-5 (5): white powder; mp 216–219 °C; $[\alpha]^{20}_D$ +12.9 (*c* 1.00, MeOH); IR (KBr) ν_{max} 3400, 1726 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1, 2, and S5; HRESIMS (negative) *m/z* 1133.5399 [calcd for $C_{54}H_{85}O_{25}$ $(M - 1)^-$, 1133.5380].

Inhibitory Activity on Induction of ICAM-1. Experimental details have been described in full in previous papers.[20,21a,b,22]

Cell Growth Inhibitory Activity to WI-38, VA-13, and HepG2 *in Vitro*. Experimental details have been described in previous papers.[18a,b,20,22,23]

Cellular Accumulation of Calcein. Experimental details have been described in previous papers.[18a,b,22,23]

Supporting Information Available: ^1H and ^{13}C data (Tables S1−S5) for new compounds **1**−**5** including HMBC and NOESY correlations and ^{13}C NMR data for known compounds **6**−**12** (Tables S6 ad S7). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The structure of **6** was known;[8] the genin of **6** is phytolaccagenin with the known absolute configuration and sugar moiety of **6** consisting of D-xylose and D-glucose. Considering the biosynthesis of an oleanane-type triterpene in the same plant, the absolute configuration of genin moieties of **1**, **2**, and **6** is the same. The only difference in their structures is the substituent at C-11. In a comparison of ^{13}C NMR spectra, δ_C values of C-2, C-3, C-4, C-1′ C-2′, and C-3′ in compounds **1** and **2** were exactly the same as those of **6**. If the sugar moiety at C-3 of **1** and **2** is L-xylose instead of D-xylose, they will show different δ_C

values. The same explanation is possible for δ_C values around the glucopyranosyl bond at C-4′ of xylopyranose and the glucopyranosyl bond at C-28. Since compounds **1** and **6** showed the same sign of their $[\alpha]_D$ values, we deduced the same absolute structure for **1** and **2** as **6**.

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