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Bioactive 3,4-*seco*-Triterpenoids from the Fruits of *Acanthopanax sessiliflorus*

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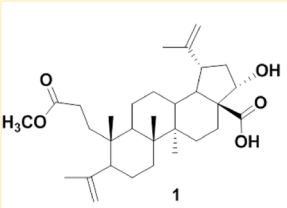
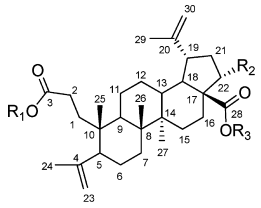
Supporting Information

ABSTRACT: Eight new 3,4-*seco*-lupane triterpenes and glycosides, acanthosessiligenins I and II (**1**, **3**) and acanthosessiliosides A–F (**2**, **4**–**8**), as well as six known 3,4-*seco*-lupane triterpenes (**9**–**14**) were isolated from an ethanolic extract of *Acanthopanax sessiliflorus* fruits. The chemical structures of **1**–**8** were determined by spectroscopic data interpretation. All isolated compounds were tested for their cytotoxicity against six human cancer cell lines and their ability to inhibit LPS-induced nitric oxide production in RAW 264.7 macrophages.

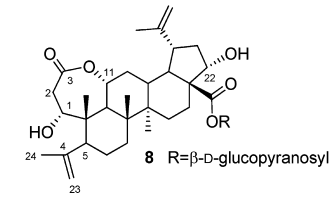


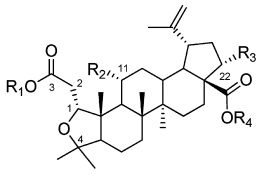
Acanthopanax sessiliflorus (Rupr. et Maxim) Seem, belonging to the plant family Araliaceae, is distributed widely in Korea, mainland China, and Japan. *Acanthopanax* species are used commonly in traditional oriental medicine to treat rheumatoid arthritis, diabetes, tumors, hypertension, and cerebrovascular diseases.[1,2] Previous phytochemical research has resulted in the isolation of lupane triterpene glycosides from the leaves and twigs of *Acanthopanax* species[3,4] and 3,4-*seco*-lupane triterpenes from the leaves of *A. divaricatus and A. senticosus*.[5,6] Triterpenoids and lignans are thought to be the active constituents of plants in this genus.[7−9] However, most phytochemical and biological studies have focused mainly on the leaves, bark, and roots of *Acanthopanax* species, and only a few studies have dealt with the fruits.

We report herein the isolation of eight new 3,4-*seco*-lupane triterpenes (**1**, **3**) and glycosides (**2**, **4**–**8**) from the fruits of *A. sessiliflorus*, together with six known compounds (**9**–**14**). The present search for bioactive constituents from Korean medicinal plants revealed that an ethanol extract of *A. sessiliflorus* fruit showed marginal cytotoxicity when evaluated against human cancer cells (data not shown). In addition, investigations of the extracts and components from *Acanthopanax* species have demonstrated previously evidence for anti-inflammatory effects by these substances.[10,11] Therefore, the isolated compounds **1**–**14** were evaluated for their cytotoxic activity against a small panel of human cancer cell lines and were also tested for their anti-inflammatory potential using lipopolysaccharide (LPS)-induced nitric oxide production inhibition in RAW 254.7 macrophage cells.



	R_1	R_2	R_3
1	CH_3	OH	H
2	H	H	β-D-glucopyranosyl





	R_1	R_2	R_3	R_4
3	CH_3	OH	H	H
4	CH_3	OH	H	β-D-glucopyranosyl
5	CH_3	OH	OH	β-D-glucopyranosyl
7	H	H	OH	β-D-glucopyranosyl

	R_1	R_2	R_3	R_4
6	β-D-glucopyranosyl	H	H	H

RESULTS AND DISCUSSION

A 70% ethanolic extract of dried *A. sessiliflorus* fruits was suspended in H_2O and extracted successively with EtOAc and *n*-BuOH. The EtOAc- and *n*-BuOH-soluble fractions were concentrated under reduced pressure to produce a residue that was subjected to multiple chromatographic steps, using Diaion HP-20, Sephadex LH-20, silica gel, and reversed-phase C_{18} silica gel, yielding compounds **1**–**14**.

Comparison of the NMR and MS data with reported values led to identification of the known compounds as 22α-

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−8[a]

position	1	2	3	4	5
1α	1.72 (m)	2.20 (m)			
1β		1.55 (m)	4.72 (dd, 2.4, 11.6)	4.84 (dd, 2.4, 11.6)	4.88 (dd, 5.2, 13.2)
2α	2.47 (m)	1.25 (m)	2.50 (dd, 8.4, 11.6)	2.68 (dd, 8.4, 11.6)	2.64 (dd, 8.0, 11.2)
2β	2.42 (m)	1.81 (m)	3.49 (dd, 2.8, 14.0)	3.65 (dd, 2.8, 14.0)	3.65 (dd, 2.4, 14.6)
9α			1.78 (d, 10.8)	1.78 (d, 10.8)	1.90 (d, 10.4)
11α	1.38 (m)	1.25 (m)	4.00 (ddd, 6.2, 10.8, 11.6)	4.08 (ddd, 6.4, 10.8, 11.8)	4.18 (m) overlap
11β					
18	2.62 (dd, 10.8, 11.2)	1.80 (dd, 9.6, 9.6)	1.71 (m)	1.81 (dd, 11.8, 11.8)	2.60 (dd, 11.2, 11.8)
19	3.66 (ddd, 4.6, 10.8, 11.2)	3.47 (ddd, 4.4, 9.6, 9.6)	3.31 (ddd, 4.4, 10.8, 11.2)	3.36 (ddd, 5.2, 10.8, 11.2)	3.58 (ddd, 4.4, 10.8, 11.2)
22α		1.72 (m)	2.08 (m)	2.10 (m)	
22β	4.80 (brd, 4.4)	1.36 (m)	1.40 (m)	1.45 (m)	4.77 (brd, 5.2)
23a	5.07 (d, 2.0)	4.93 (brs)	1.09 (s)	1.16 (s)	1.15 (s)
23b	4.83 (d, 2.0)	4.75 (brs)			
24	1.72 (s)	1.62 (s)	1.37 (s)	1.37 (s)	1.38 (s)
25	1.12 (s)	1.00 (s)	1.29 (s)	1.29 (s)	1.31 (s)
26	1.21 (s)	1.06 (s)	0.91 (s)	1.14 (s)	1.21 (s)
27	0.80 (s)	0.66 (s)	1.05 (s)	1.13 (s)	1.25 (s)
29	2.03 (s)	1.77 (s)	1.69 (s)	1.69 (s)	1.94 (s)
30a	4.90 (brs)	4.79 (brs)	4.77 (brs)	4.78 (brs)	4.92 (brs)
30b	4.79 (brs)	4.75 (brs)	4.61 (brs)	4.62 (brs)	4.67 (brs)
OCH_3	3.65 (s)		3.61 (s)	3.61 (s)	3.60 (s)
1′		6.32 (d, 8.0)		6.39 (d, 8.0)	6.41 (d, 8.0)
2′		4.11 (dd, 8.0, 8.0)		4.16 (dd, 9.2, 9.2)	4.13 (dd, 7.6, 8.0)
3′		4.27 (dd, 7.6, 8.0)		4.27 (dd, 9.2, 9.2)	4.22 (dd, 7.6, 7.6)
4′		4.33 (dd, 7.6, 8.0)		4.34 (dd, 9.2, 9.2)	4.28 (dd, 7.6, 7.6)
5′		4.00 (m)		4.01 (m)	4.03 (m)
6′α		4.41 (dd, 2.0, 12.0)		4.43 (dd, 2.0, 12.0)	4.43 (dd, 2.8, 12.0)
6′β		4.32 (dd, 4.8, 12.0)		4.39 (dd, 4.4, 12.0)	4.35 (dd, 4.8, 12.0)

position	6	7	8
1α			
1β	4.28 (dd, 4.4, 12.4)	4.47 (dd, 3.6, 11.2)	3.70 (dd, 3.2, 7.6)
2α	2.54 (dd, 2.4, 14.0)	2.56 (m)	2.81 (brd, 14.4)
2β	2.75 (m)	2.80 (m)	3.08 (dd, 8.4, 14.4)
9α	1.68 (m)	1.80 (m)	2.74 (d, 9.2)
11α	1.82 (m)	1.20 (m)	4.54 (ddd, 8.8, 9.2, 9.2)
11β			
18	1.77 (dd, 9.6, 10.8)	2.70 (dd, 10.8, 11.2)	2.53 (dd, 11.2, 11.6)
19	3.47 (ddd, 5.6, 10.8, 11.2)	3.57 (ddd, 4.4, 10.8, 11.2)	3.53 (ddd, 4.8, 11.2, 11.6)
22α	2.25 (m)		
22β	1.59 (m)	4.77 (brd, 4.4)	4.75 (brd, 4.4)
23a	1.08 (s)	1.11 (s)	5.10 (brs)5.00 (brs)
23b			
24	1.30 (s)	1.39 (s)	1.85 (s)
25	0.93 (s)	1.06 (s)	0.97 (s)
26	0.99 (s)	1.17 (s)	1.14 (s)
27	1.04 (s)	1.20 (s)	1.13 (s)
29	1.82 (s)	1.98 (s)	1.90 (s)
30a	4.96 (brs)	5.00 (brs)	4.99 (brs)
30b	4.80 (brs)	4.80 (brs)	4.66 (brs)
OCH_3			
1′	6.35 (d, 8.0)	6.47 (d, 8.0)	6.44 (d, 8.0)
2′	4.11 (dd, 8.0, 8.4)	4.19 (dd, 8.0, 8.4)	4.18 (dd, 8.0, 8.4)
3′	4.27 (dd, 8.4, 8.8)	4.32 (dd, 8.4, 8.4)	4.30 (dd, 8.4, 8.4)
4′	4.30 (dd, 8.8, 8.8)	4.37 (dd, 8.4, 8.4)	4.35 (dd, 8.4, 8.4)
5′	4.01 (m)	4.05 (m)	4.06 (m)
6′α	4.43 (dd, 2.8, 12.0)	4.46 (dd, 2.0, 12.0)	4.47 (brd, 11.2)
6′β	4.35 (dd, 4.8, 12.0)	4.38 (dd, 4.4, 12.0)	4.34 (dd, 3.6, 11.2)

[a] ^{1}H NMR data were measured in pyridine-d_5 at 400 MHz. Chemical shift (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses. The assignments were based on DEPT, COSY, NOESY, HSQC, and HMBC experiments.

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Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−8[a]

position	1	2	3	4	5	6	7	8
1	34.9	31.5	86.6	87.2	87.2	84.5	85.5	70.5
2	28.8	30.3	38.0	38.7	38.3	38.1	38.4	38.8
3	174.2	178.6	173.5	173.3	173.4	170.9	175.8	173.1
4	147.9	147.4	79.3	79.4	79.3	81.6	82.4	147.7
5	50.5	50.0	56.2	56.1	56.1	56.1	56.2	49.5
6	26.2	26.4	18.1	18.8	18.8	19.0	18.8	25.1
7	33.3	331	34.9	35.5	35.5	34.6	34.4	32.3
8	39.7	39.5	42.3	42.9	42.9	43.3	43.2	41.7
9	41.3	41.0	48.9	48.9	49.0	42.7	42.7	44.1
10	43.4	43.2	46.3	47.0	47.0	47.9	47.7	44.2
11	22.1	21.9	67.2	67.7	67.8	23.8	23.8	75.3
12	25.1	24.9	36.9	37.0	37.0	25.7	25.5	33.5
13	38.6	38.5	37.2	37.7	37.4	38.8	38.6	35.0
14	40.9	40.7	42.1	42.8	42.8	41.6	41.6	42.1
15	30.1	30.0	29.9	29.7	29.8	30.6	29.8	28.9
16	27.2	31.9	32.3	31.0	26.7	31.3	26.7	26.6
17	62.9	56.6	55.4	57.0	63.0	56.6	62.9	62.9
18	44.3	49.7	48.3	49.6	44.1	49.8	44.2	44.1
19	47.9	47.7	47.0	47.4	47.5	47.8	47.6	47.7
20	151.7	151.1	150.5	150.5	150.6	151.4	151.4	150.5
21	42.3	32.7	30.6	30.5	41.9	32.9	41.7	41.6
22	75.6	37.3	36.3	36.8	74.8	37.6	74.9	74.8
23	113.7	114.0	24.2	25.0	24.8	24.6	24.7	113.8
24	23.6	23.6	32.0	32.4	32.6	32.6	32.7	23.4
25	20.5	20.2	18.5	19.3	19.1	18.8	19.2	19.0
26	16.5	16.3	17.3	18.0	17.9	17.0	17.0	17.8
27	15.0	14.9	14.6	15.3	15.2	15.0	14.9	13.8
28	178.8	174.8	179.0	174.8	174.7	178.8	174.8	174.8
29	19.5	19.6	19.1	19.6	19.3	19.6	19.3	18.7
30	110.4	110.0	109.7	110.1	110.7	110.0	110.6	111.1
OCH_3	51.5		51.1	51.2	51.0			
1′		95.5		95.5	95.6	96.3	95.5	95.5
2′		74.1		74.4	74.3	74.5	74.3	74.3
3′		78.4		78.7	78.7	78.3	78.8	78.8
4′		71.0		71.2	71.2	71.2	71.1	71.0
5′		79.3		79.5	79.4	79.4	79.7	79.5
6′		62.2		62.3	62.3	62.4	62.1	62.1

[a] ^{13}C NMR data were measured in pyridine-d_5 at 100 MHz. The assignments were based on DEPT, COSY, NOESY, HSQC, and HMBC experiments.

hydroxychiisanogenin (**9**),[12] 3,4-*seco*-lupan-20(30)-ene-3,28-dioic acid (**10**),[13] (1*R*)-1,4-epoxy-11α,22α-hydroxy-3,4-*seco*-lupan-20(30)-ene-3,28-dioic acid (**11**),[14] (+)-divaroside (**12**),[15] chiisanoside (**13**),[5] and 22α-hydroxychiisanoside (**14**).[12]

Compounds **1**−**8** showed bands at 3462 to 3342, 1746 to 1710, and 1665 to 1640 cm^{-1} in the FT-IR spectrum, suggesting the presence of hydroxy group, carbonyl group, and double-bond absorptions. Of these, compound **1** was obtained as a white, amorphous powder. The molecular formula was determined to be $C_{31}H_{48}O_5$ from the pseudomolecular ion peak $[M - H]^-$ at m/z 499.3401 (calcd for $C_{31}H_{47}O_5$, 499.3423) in the negative HRFABMS. In the ^{1}H NMR spectrum (Table 1) the observation of signals for two allyl methyl protons [δ_H 1.72 (H-24) and 2.03 (H-29)] located at sp^2 carbons and four olefinic methine protons at δ_H 5.07 (J = 2.0 Hz, H-23a), 4.83 (J = 2.0 Hz, H-23b), 4.90 (brs), and 4.79 (brs), of which the chemical shifts and small coupling constants (or broad singlet) were typical of two exomethylene units, confirmed the presence of two isopropenyl moieties. Also, signals for three tertiary methyl protons [δ_H 1.21 (H-26), 1.12 (H-25), 0.80 (H-27)], an oxygenated methine proton [δ_H 4.80 (brd, J = 4.4 Hz, H-22)], and a methoxy proton (δ_H 3.65) were observed. The ^{13}C NMR spectrum of **1** (Table 2) supported by DEPT experiments indicated the presence of 31 carbons including two carbonyl carbons [δ_C 174.2 (C-3) and 178.8 (C-28)], two olefinic quaternary carbons [δ_C 151.7 (C-20), 147.9 (C-4)], two exomethylene carbons [δ_C 113.7 (C-23), 110.4 (C-30)], an oxygenated methine carbon [δ_C 75.6 (C-22)], a methoxy carbon (δ_C 51.5), five methyl carbons [(δ_C 23.6 (C-24), 20.5 (C-25), 19.5 (C-29), 16.5 (C-26), 15.0 (C-27)], and 18 other carbon signals. Several cross-peaks in the ^{1}H−^{1}H COSY spectrum confirmed a key connection among the proton signals (Figure 1). The HMBC spectrum showed crucial long-range correlations between H-23a, H-23b/C-5; H-2a, H-2b/C-3; and H-1a/C-3 (Figure 1). On further analysis of the HSQC and DEPT 135 spectra of **1**, the assignments of the proton and carbon NMR signals (Tables 1 and 2) were confirmed unambiguously. Therefore, **1** was assigned as a 3,4-*seco*-lupane-type triterpenoid. In addition, a long-range correlation

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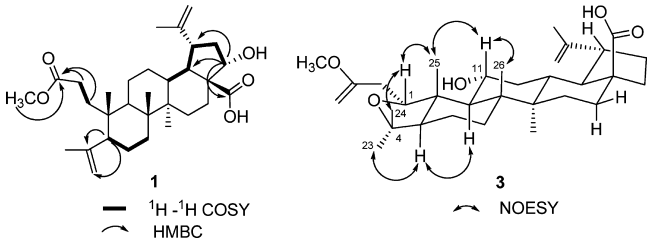

Figure 1. Key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of compounds **1** and **3**.

between the signals of the methoxy protons (δ_H 3.65) and the C-3 carbonyl carbon (δ_C 174.2) was also evident. The coupling constant between H-22 and H-21 was 4.4 Hz, indicating that OH-22 is α-oriented.[12] Therefore, the structure of compound **1** was determined as 22α-hydroxy-3,4-*seco*-lupa-4(23),20(30)-diene-3,28-dioic acid 3-methyl ester, which has previously been unreported, and this compound was named acanthosessiligenin I.

Compound **2** was isolated as a white powder, and its molecular formula was established as $C_{36}H_{56}O_9$ from the pseudomolecular ion peak $[M + H]^+$ at m/z 633.3637 (calcd for $C_{36}H_{57}O_9$, 633.4002) in the positive HRFABMS. Its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were similar to those of **1**, with the exception of proton and carbon resonances for an additional sugar moiety and the lack of a methoxy group and an oxygenated methine moiety at the C-3 and C-22 position, respectively. The quaternary carbon signal for C-17 (δ_C 56.6) shifted 6.3 ppm upfield compared with **1** and 22α-hydroxychiisanoside (**14**),[12] suggesting that C-22 of **2** is a methylene group. As for the monosaccharide unit, an anomeric proton signal at δ_H 6.32 (d, J = 8.0 Hz) and a carbon signal at δ_C 95.5 (C-1′) as well as the oxygenated methine and methylene carbon signals at δ_C 79.3 (C-5′), 78.4 (C-3′), 74.1 (C-2′), 71.0 (C-4′), and 62.2 (C-6′) suggested the presence of a β-glucopyranosyl group. The connectivity between the glucopyranosyl unit (C-1′) and the C-28 of the aglycone was verified by the presence of a cross-peak between δ_H 6.32 (H-1′) and δ_C 174.8 (C-28) in the HMBC spectrum as well as the chemical shift of the anomeric carbon signal (δ_C 95.5). Therefore, the structure of **2** (acanthosessilioside A) was determined to be 3,4-*seco*-lupa-4(23),20(30)-diene-3,28-dioic acid 28-*O*-β-D-glucopyranoside.

Compound **3** was isolated as white, needle-like crystals. The positive HRFABMS of **3** showed a $[M + H]^+$ ion peak at m/z 517.3307, in accordance with a pseudomolecular ion of $C_{31}H_{49}O_6$ (calcd for $C_{31}H_{49}O_6$, 517.3529). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) exhibited signals for five tertiary methyl groups (δ_H 1.37, 1.29, 1.09, 1.05, 0.91), an allyl methyl group (δ_H 1.69), an exomethlyene group (δ_H 4.77, 4.61) due to an isopropenyl moiety, two oxygenated methine groups [δ_H 4.72 (dd, J = 2.4, 11.6 Hz, H-1), δ_C 86.6 (C-1); δ_H 4.00 (ddd, J = 6.2, 10.8, 11.6 Hz, H-11), δ_C 67.2 (C-11)], a methoxy group (δ_H 3.61, δ_C 51.1), and two carbonyl groups [δ_C 173.5 (C-3), 179.0 (C-28)]. When compared with a previously reported compound, isochiisanoside,[12] **3** was found to lack a sugar moiety at the C-28 position but to possess an additional methoxy group. The oxygenated methine proton (H-11) of **3** appeared at δ_H 4.00 (ddd, $J_{11,12eq}$ = 6.2 Hz, $J_{11,12ax}$ = 11.6 Hz, $J_{9,11}$ = 10.8 Hz), coupled with H-9 [δ_H 1.78 (d, J = 10.8 Hz)]. These data indicated a diaxial arrangement between H-9 and H-11 and an equatorial confirmation of the hydroxy group at C-11. Furthermore, the stereostructure of **3** was confirmed by a NOESY experiment (Figure 1), which showed a correlation between H-1 and H_3-24, H_3-25, as well as between H-11 and H_3-25, H_3-26. These NOE correlations indicated that the oxygen at C-1 is β-oriented and that the hydroxy at C-11 is α-configured. Also, the long-range correlations between the methoxy protons signal (δ_H 3.61) and the carbonyl carbon signal at C-3 (δ_C 173.5) in the HMBC spectrum revealed that the methoxy group is affixed to C-3. Consequently, the structure of **3** (acanthosessiligenin II) was determined as (1*R*)-1,4-epoxy-11α-hydroxy-3,4-*seco*-lup-20(30)-ene-3,28-dioic acid 3-methyl ester.

Compound **4**, a white powder, showed a pseudomolecular ion peak $[M + H]^+$ at m/z 679.4003 in the positive HRFABMS, which was 162 mass units larger than that of **3** and consistent with a pseudomolecular formula of $C_{37}H_{59}O_{11}$ (calcd for $C_{37}H_{59}O_{11}$, 679.4057). A comparison of the ^{1}H and ^{13}C NMR spectra showed that they are quite similar to those of **3** except for the presence of proton and carbon resonances for an additional β-glucopyranosyl group (Tables 1 and 2). This finding was confirmed by acid hydrolysis of **4**, which yielded the same aglycone [TLC, R_f 0.55 (RP-18 F_{254s}), acetone−H_2O (4:1)] as that of **3** and D-glucose [TLC, R_f 0.40 (silica gel F_{254}), $CHCl_3$−MeOH−H_2O (65:35:10)]. The glucose moiety was determined to be attached to C-28 by observation of the glycosylation effect of the ^{13}C NMR resonance of C-28 relative to that in **3** (δ_C 174.8 in **4** vs δ_C 179.0 in **3**) and from the long-range correlations of H-1′ (δ_H 6.39, d, J = 8.0 Hz) with C-28 (δ_C 174.8) observed in the HMBC spectrum. Therefore, the structure of **4** (acanthosessilioside B) was determined as (1*R*)-1,4-epoxy-11α-hydroxy-3,4-*seco*-lup-20(30)-ene-3,28-dioic acid 3-methyl ester 28-*O*-β-D-glucopyranoside.

Compound **5** was isolated as a white powder, and its molecular formula was established as $C_{37}H_{58}O_{12}$ from the pseudomolecular ion $[M + H]^+$ at m/z 695.3936 (calcd for $C_{37}H_{59}O_{12}$, 695.4006) in the positive HRFABMS. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **5** were similar to those of **4** except that the methylene signals due to C-22 in **4** were replaced by those of an oxygenated methine (δ_H 4.77 and δ_C 74.8) in **5**. The molecular weight of **5** was 16 Da more than that of **4**, indicating the presence of an additional hydroxy group. In **5**, the chemical shifts of the carbon signals of C-17 (δ_C 63.0) and C-21 (δ_C 41.9) were shifted downfield by 6.0 and 11.4 ppm, respectively, and that of C-18 (δ_C 44.1) was shifted upfield by 5.5 ppm when compared with **4** and isochiisanoside,[12] suggesting that C-22 is hydroxylated. This conclusion was supported by the HMBC spectrum, which showed a long-range correlation between the proton signal of H-22 (δ_H 4.77) and the carbon signals of C-18 (δ_C 44.1), C-19 (δ_C 47.5), and C-28 (δ_C 174.7). The coupling constant between H-22 and H-21 was 5.2 Hz, indicating that OH-22 is α-oriented.[12,14] Consequently, the structure of compound **5** (acanthosessilioside C) was determined as (1*R*)-1,4-epoxy-11α,22α-dihydroxy-3,4-*seco*-lup-20(30)-ene-3,28-dioic acid 3-methyl ester 28-*O*-β-D-glucopyranoside.

Compound **6**, a white powder, showed a pseudomolecular ion peak $[M - H]^-$ at m/z 647.3744 in the negative HRFABMS, and the mass spectrometric data were consistent with the pseudomolecular formula of $C_{36}H_{55}O_{10}$ (calcd for $C_{36}H_{55}O_{10}$, 647.3795). The ^{13}C NMR and DEPT spectra indicated the presence of 36 carbons, including six methyls, nine methylenes, six methines, eight quaternary carbons, and one glycosyl moiety (Tables 1 and 2). The ^{13}C NMR signals for

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C-9 and C-12 (δ_C 42.7 and 25.7) were shifted upfield by 6.3 and 11.3 ppm, respectively, and those of C-10 and C-13 shifted downfield by 0.9 and 1.4 ppm, respectively, when compared with the ^{13}C NMR signals of **5** and isochiisanoside.[12] This indicated that C-11 of **6** is not oxygen-bearing. When compared with a previously isolated compound, 3,4-*seco*-lupan-20(30)-ene-3,28 dioic acid (**10**),[13] compound **6** was determined to be a monoglycoside of **10**. The proton and carbon signals due to the sugar moiety suggested the presence of a β-glucopyranosyl group (Tables 1 and 2). The correlation between δ_H 6.35 (H-1′) and δ_C 170.9 (C-3) in the HMBC spectrum and the chemical shift of the anomeric carbon signal (δ_C 96.3) supported the presence of a glucopyranosyl group at C-3. Therefore, the chemical structure of compound **6** (acanthosessilioside D) was determined as (1*R*)-1,4-epoxy-3,4-*seco*-lup-20(30)-ene-3,28-dioic acid 3-*O*-β-D-glucopyranoside.

Compound **7** was isolated as a white powder, and its molecular formula was established as $C_{36}H_{56}O_{11}$ from the pseudomolecular ion peak $[M + H]^+$ at *m/z* 665.3994 (calcd for $C_{36}H_{57}O_{11}$, 665.3901) in the positive HRFABMS. The ^1H and ^{13}C NMR spectra of **7** were found to be similar to those of **6** (Tables 1 and 2), with the exception of the proton and carbon resonances for an oxygenated methine group (δ_H 4.77, brd, J = 4.4 Hz and δ_C 74.9) in **7**. A hydroxy group could be positioned at C-22 from the correlation between the proton signal of H-22 and the carbon signals of C-18 (δ_C 44.2), C-19 (δ_C 47.6), and C-28 (δ_C 174.8) in the HMBC spectrum. The J value and the configuration of H-22 were in good agreement with those of **5** and other reported data.[12,14] Furthermore, the molecular weight of **7** was 16 Da more than **6**, indicating the presence of an additional hydroxy group. The glucose moiety was deduced to be attached to C-28 via a glycosidic linkage, from the glycosidation-induced shift of the ^{13}C NMR resonance of C-28 relative to that in **6** (δ_C 178.8 in **6** vs δ_C 174.8 in **7**) and from a long-range correlation of H-1′ (δ_H 6.47, d, J = 8.0 Hz) with C-28 (δ_C 174.8) observed in the HMBC spectrum. Therefore, the structure of compound **7** (acanthosessilioside E) was determined as (1*R*)-1,4-epoxy-22α-hydroxy-3,4-*seco*-lup-20(30)-ene-3,28-dioic acid 28-*O*-β-D-glucopyranoside.

Compound **8** was isolated as a white powder, and its molecular formula was established as $C_{36}H_{54}O_{11}$ from the pseudomolecular ion peak $[M + H]^+$ at *m/z* 663.3753 (calcd for $C_{36}H_{55}O_{11}$, 663.3744) in the positive HRFABMS. The ^1H and ^{13}C NMR spectra of **8** (Tables 1 and 2) suggested it to be a monoglycoside of 22α-hydroxychiisanogenin (**9**).[12] The sugar moiety was determined to be β-glucopyranoside from a hemiacetal proton signal at δ_H 6.44 (d, J = 8.0 Hz) and a hemiacetal carbon signal, four oxygenated methine carbon signals, and an oxygenated methylene carbon signal (Table 2). The signal at δ_C 95.5 suggested that **8** has a 28-*O*-glycosidic linkage through an ester bond, which was confirmed from a long-range correlation of H-1′ with C-28 (δ_C 174.8) observed in the HMBC spectrum. Thus, the structure of compound **8** (acanthosessilioside F) was determined as (1*R*)-1α,11α,22α-trihydroxy-3,4-*seco*-lupa-4(23),20(30)-diene-3,28-dioic acid 3,11-lactone 28-*O*-β-D-glucopyranoside.

The monosaccharide obtained after aqueous acid hydrolysis of each glycoside (**2**, **4**−**8**) was identified as glucose by TLC comparison with an authentic sample [TLC, R_f 0.40 (silica gel F_{254}), $CHCl_3$−MeOH−H_2O (65:35:10)]. The absolute configuration was determined to be D based on GC analysis of a chiral derivative of the monosaccharide obtained by hydrolysis of each compound (see Experimental Section). The relatively large coupling constant (J = 8.0 Hz) of the anomeric proton signals in the ^1H NMR spectra (Table 1) of these glycosides suggested the β-configuration of the glucopyranosyl moiety in each case.

All isolated compounds (**1**−**14**) were tested for their biological activity. All compounds were inactive when tested against a small cancer cell line panel (IC_{50} values <10 μM). As shown in Table 3, compounds **1**−**4** and **6** showed moderate potencies in inhibiting NO production and had no influence on cell viability. All other compounds were inactive (IC_{50} > 50 μM) against LPS-induced NO production in RAW 264.7 macrophages.

Table 3. Inhibitory Effects of Compounds 1−14 against LPS-Induced NO Production in RAW264.7 Macrophage Cells

compound	IC_{50} (μM)[a]	cell viability (%)[b]
1	11.3	88.8 ± 3.7
2	18.3	93.5 ± 2.0
3	47.0	91.1 ± 3.4
4	20.6	93.0 ± 5.7
6	11.9	95.2 ± 1.2
aminoguanidine[c]	6.5	84.6 ± 2.5

[a]The IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated RAW 264.7 macrophage cells. Compounds **5** and **7**−**14** were inactive (IC_{50} > 50 μM). [b]Cell viability was expressed as a percentage (%) of the LPS-only treatment group. [c]Positive control. The results are averages of three independent experiments, and the data are expressed as means ± SD.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained using a Fisher-John's melting point apparatus with a microscope. Optical rotations were measured on a JASCO P-1010 digital polarimeter. ^1H, ^{13}C, and 2D NMR spectra were recorded on a Varian Unity INOVA AS 400 FT-NMR instrument, and chemical shifts are given in δ (ppm) based on tetramethylsilane (TMS) as internal standard. IR spectra were run on a Perkin-Elmer Spectrum One FT-IR spectrometer. HRFABMS were obtained using a JEOL JMS-700 mass spectrometer. A Shimadzu gas chromatograph (GC-14B) equipped with an on-column injection system and flame ionization detector (FID) was used. Silica gel 60 (Merck, 230−400 mesh), LiChroprep RP-18 (Merck, 40−63 μm), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC). Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for analytical thin-layer chromatography. Spots were visualized by spraying with 10% aqueous H_2SO_4 solution, followed by heating.

Plant Material. The fruits of *A. sessiliflorus* were obtained in August 2009 from the Jeongseon Agricultural Extension Center, Jeongseon, Korea, and were identified by Prof. Dae-Keun Kim, College of Pharmacy, Woo Suk University, Jeonju, Korea. A voucher specimen (KHU090809) is preserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Extraction and Isolation. The air-dried fruits of *A. sessiliflorus* (10 kg) were powdered and extracted three times for 24 h with 36 L of aqueous 70% EtOH at room temperature. After concentration in vacuo, the EtOH extract (2012 g) was suspended in H_2O (3 L) and then partitioned successively with EtOAc (3 L × 3) and *n*-BuOH (3 L), followed by concentration, to give EtOAc (E, 118 g), *n*-BuOH (B, 284 g), and water (1610 g) fractions. Fraction E (100 g) was subjected to silica gel CC (15 × 21 cm) using a gradient of $CHCl_3$−MeOH (15:1 → 10:1 → 5:1 → 3:1 → 1:1, 2.8 L each) to yield 14 fractions (E1 to E14). Fraction E3 [36.3 g, elution volume/total volume (V_e/V_t) 0.15−0.33] was subjected to silica gel CC [6 × 16 cm, $CHCl_3$−EtOAc (7:1, 5.5 L)] to give five subfractions (E3-1 to E3-5). CC [silica

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gel (3.5 × 16 cm), *n*-hexane−EtOAc (1:1, 3 L)] of subfraction E3-3 (1.80 g, V_e/V_t 0.34−0.53) gave 19 subfractions (E3-3-1 to E3-3-19). Subfraction E3-3-7 (184 mg, V_e/V_t 0.30−0.41) was separated by CC [RP-18 (3.5 × 5.5 cm), acetone−H_2O (3:2, 1.6 L)] to give compound **3** [19.7 mg, V_e/V_t 0.46−0.65, TLC (RP-18 F_{254s}) R_f 0.55, acetone−H_2O (4:1)] and compound **10** [12.2 mg, V_e/V_t 0.87−0.92, TLC (RP-18 F_{254s}) R_f 0.25, acetone−H_2O (4:1)]. Subfraction E3-3-9 (95 mg, V_e/V_t 0.49−0.53) was chromatographed [RP-18 (3.5 × 6.5 cm), acetone−MeOH−H_2O (1:1:4, 0.8 L)] to give compound **9** [38.9 mg, V_e/V_t 0.52−0.76, TLC (RP-18 F_{254s}) R_f 0.40, acetone−MeOH−H_2O (1:1:1)]. Fraction E8 (8.98 g, V_e/V_t 0.59−0.67) was fractionated using silica gel CC [4 × 12 cm, $CHCl_3$−MeOH−H_2O (16:3:1 → 13:3:1, each 3.7 L)] and gave nine subfractions (E8-1 to E8-9). Subfraction E8-4 (1.85 g, V_e/V_t 0.45−0.58) was purified using CC [RP-18 (3.5 × 6.5 cm), MeOH−H_2O (3:1, 1.2 L)] and gave compound **2** [93 mg, V_e/V_t 0.84−0.98, TLC (RP-18 F_{254s}) R_f 0.40, MeOH−H_2O (5:1)] and compound **6** [13.5 mg V_e/V_t 0.65−0.70, TLC (RP-18 F_{254s}) R_f 0.45, MeOH−H_2O (5:1)]. Subfraction E8-5 (1.22 g, V_e/V_t 0.59−0.68) was fractionated using Sephadex LH 20 CC [3 × 50 cm, MeOH−H_2O (4:1, 1.8 L)] and yielded five subfractions (E8-5-1 to E8-5-5). Purification of subfraction E8-5-1 (280 mg, V_e/V_t 0.01−0.25) using CC [RP-18 (3 × 6 cm), MeOH−H_2O (1:1, 0.6 L)] gave compound **11** [35 mg, V_e/V_t 0.77−0.85, TLC (RP-18 F_{254s}) R_f 0.40, MeOH−H_2O (2:1)]. Fraction E9 (5.80 g, V_e/V_t 0.68−0.72) was fractionated using silica gel CC [5 × 18 cm, $CHCl_3$−EtOH−H_2O (16:3:1 → 13:3:1 → 10:3:1, each 3.2 L)] and gave four subfractions (E9-1 to E9-4). Subfraction E9-1 (1.25 g, V_e/V_t 0.01−0.30) was separated by RP-18 (3 × 6 cm) CC using MeOH−H_2O (3:1, 1.5 L) as eluent and was further purified by RP-18 CC (2.5 × 5 cm), eluting with MeOH−H_2O (2:1), to give compound **4** [23 mg, V_e/V_t 0.54−0.61, TLC (RP-18 F_{254s}) R_f 0.30, MeOH−H_2O (3:1)]. Subfraction E9-2 (2.45 g, V_e/V_t 0.31−0.68) was chromatographed over RP-18 (5 × 5.5 cm), eluting with MeOH−H_2O (1:1 → 2:1, each 1.8 L), to provide 11 subfractions (E9-2-1 to E-9-2-11). Subfraction E9-2-7 (140 mg, V_e/V_t 0.66−0.78) was purified over silica gel (3 × 15 cm) and eluted with $CHCl_3$−MeOH (3:1, 0.8 L) to give compound **1** [21 mg, V_e/V_t 0.22−0.32, TLC (silica gel F_{254}) R_f 0.55, $CHCl_3$−MeOH−H_2O (13:3:1)]. Fraction E10 (6.75 g, V_e/V_t 0.72−0.78) was fractionated using silica gel CC [7 × 15 cm, $CHCl_3$−MeOH−H_2O (17:3:1 → 15:3:1 → 13:3:1, each 3.2 L)] and gave 10 subfractions (E10-1 to E10-10). Subfraction E10-6 (624 mg, V_e/V_t 0.75−0.82) was separated by RP-18 (3.5 × 9 cm) CC, eluting with MeOH−H_2O (2:3, 1.7 L), to obtain compound **7** [8 mg, V_e/V_t 0.78−0.81, TLC (RP-18 F_{254s}) R_f 0.50, MeOH−H_2O (3:1)]. Subfraction E10-4 (1.64 g, V_e/V_t 0.35−0.60) was chromatographed over a RP-18 column (4.5 × 10 cm), eluted with MeOH−H_2O (1:1.5, 2 L), and further purified by RP-18 (2.5 × 7 cm) CC [E10-4-14 (34 mg, V_e/V_t 0.76−0.81)], using MeOH−H_2O (2:1, 0.4 L) as eluent, to afford compound **5** [10 mg, V_e/V_t 0.50−1.00, TLC (RP-18 F_{254s}) R_f 0.30, MeOH−H_2O (2:1)]. Fraction E12 (10.56 g, V_e/V_t 0.84−0.91) was fractionated using silica gel CC [$CHCl_3$−EtOH−H_2O, 17:3:1 → 12:3:1 → 9:3:1 (each 3.5 L)] and gave 25 subfractions (E12-1 to E12-25). Subfraction E12-6 (559 mg, V_e/V_t 0.23−0.28) was subjected to RP-18 CC [3.5 × 4.5 cm, MeOH−H_2O (1:3 → 1:2 → 1:1, each 0.8 L)] to give 18 fractions (E12-6-1 to E12-6-18) and was further purified by RP-18 CC [E12-6-12 (115 mg, V_e/V_t 0.54−0.70)], using EtOH−MeOH−H_2O (1:1:3, 2.4 L) as eluent, to yield compound **8** [12 mg, V_e/V_t 0.50−0.60, TLC (RP-18 F_{254s}) R_f 0.60, EtOH−MeOH−H_2O (1:1:1)].

Fraction B was chromatographed on a column prepared with a highly porous polymer, Diaion HP-20 (12 × 45 cm), and successively eluted with H_2O and MeOH to give two fractions (B1 and B2). Fraction B2 (73.40 g) was subjected to silica gel (12 × 15 cm) CC using a gradient of $CHCl_3$−MeOH−H_2O [7:3:1 (8 L) → 65:35:10 (12 L)] to yield 11 fractions (B2-1 to B2-11). Fraction B2-4 (3.50 g, V_e/V_t 0.31−0.36) was subjected to RP-18 (5 × 7 cm) CC elution with MeOH−H_2O [1.5:1 (3.2 L) → 2:1 (2.8 L) → 4:1 (3.6 L)] to give six subfractions (B2-4-1 to B2-4-6). Subfraction B2-4-1 (913 mg, V_e/V_t 0.01−0.34) was purified over RP-18 (4 × 7.5 cm) CC, eluting with MeOH−H_2O (3:2, 2.2 L), to give compound **13** [250 mg, V_e/V_t 0.34−0.56, TLC (RP-18 F_{254s}) R_f 0.60, MeOH−H_2O (2:1)] and compound **12** [35 mg, V_e/V_t 0.87−0.92, TLC (RP-18 F_{254s}) R_f 0.25, MeOH−H_2O (2:1)]. Fraction B2-7 (4.95 g, V_e/V_t 0.45−0.52) was chromatographed over RP-18 (4 × 9 cm), eluting with MeOH−H_2O (1:1, 4 L), and gave 10 subfractions (B2-7-1 to B2-7-15). Subfraction B2-7-4 (1.05 g, V_e/V_t 0.25−0.51) was separated by RP-18 (4 × 7.5 cm) CC, eluting with MeOH−H_2O (1:1, 1.8 L), to obtain compound **14** [350 mg, V_e/V_t 0.32−0.60, TLC (RP-18 F_{254s}) R_f 0.55, MeOH−H_2O (2:1)].

Acanthosessiligenin I (**1**): white, amorphous powder; mp 230−232 °C; $[\alpha]^{20}_D$ −24.1 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3360, 1732, 1648 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; negative HRFABMS *m/z* 499.3401 $[M - H]^-$ (calcd for $C_{31}H_{47}O_5$, 499.3423).

Acanthosessilioside A (**2**): white powder; mp 242−243 °C; $[\alpha]^{20}_D$ −70.5 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3345, 1744, 1640 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRFABMS *m/z* 633.3956 $[M + H]^+$ (calcd for $C_{36}H_{57}O_9$, 633.4002).

Acanthosessiligenin II (**3**): white needles; mp 236−237 °C; $[\alpha]^{20}_D$ −18.2 (*c* 0.7, MeOH); IR (CaF_2 window) ν_{max} 3350, 1740, 16548 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRFABMS *m/z* 517.3307 $[M + H]^+$ (calcd for $C_{31}H_{49}O_6$, 517.3529).

Acanthosessilioside B (**4**): white powder; mp 248−250 °C; $[\alpha]^{20}_D$ +34.6 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3342, 1746, 1648 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRFABMS *m/z* 679.4003 $[M + H]^+$ (calcd for $C_{37}H_{59}O_{11}$, 679.4057).

Acanthosessilioside C (**5**): white powder; mp 252−253 °C; $[\alpha]^{20}_D$ +66.8 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3351, 1741, 1651 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRFABMS *m/z* 695.4006 $[M + H]^+$ (calcd for $C_{37}H_{59}O_{12}$, 695.4006).

Acanthosessilioside D (**6**): white powder; mp 247−248 °C; $[\alpha]^{20}_D$ +21.0 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3462, 1710, 1641 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; negative HRFABMS *m/z* 647.3744 $[M - H]^-$ (calcd for $C_{36}H_{55}O_{10}$, 647.3795).

Acanthosessilioside E (**7**): white powder; mp 255−256 °C; $[\alpha]^{20}_D$ −45.2 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3420, 1714, 1645 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRFABMS *m/z* 665.3892 $[M + H]^+$ (calcd for $C_{36}H_{57}O_{11}$, 665.3901).

Acanthosessilioside F (**8**): white powder; mp 259−260 °C; $[\alpha]^{20}_D$ +19.2 (*c* 0.5, MeOH); IR (CaF_2 window) ν_{max} 3380, 1740, 1655 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRFABMS *m/z* 663.3753 $[M + H]^+$ (calcd for $C_{36}H_{55}O_{11}$, 663.3744).

Acid Hydrolysis of 2 and 4−8 and Determination of the Absolute Configuration of the Monosaccharide Components. Each compound (5 mg) was hydrolyzed with 2 mL of 2 N HCl in H_2O for 6 h at 80 °C, followed by neutralization with 2 mL of 2 N NaOH in H_2O and then extracted with $CHCl_3$. The aqueous layer was concentrated under a vacuum to give a residue of the sugar fraction. The residue was dissolved in pyridine (100 μL), and then 0.1 M L-cysteine methyl ester hydrochloride (150 μL) was added. After reacting at 60 °C for 90 min, the reaction mixture was dried under a vacuum. For derivatization, 100 μL of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide was added, and the mixture incubated at 37 °C for 30 min. Then, the mixture was subjected to GC analysis under the following conditions: capillary column, DB-5 (30 m × 0.32 mm × 0.25 μm); detector, FID; detector temperature, 280 °C; injector temperature, 250 °C; carrier, N_2 gas (20.4 mL/min); oven temperature, 170−250 °C with a rate of 5 °C/min, with 1 μL of each sample injected directly into the inject port (splitless mode). By comparing the retention time (t_R) of the monosaccharide derivative with the standard sample (D-glucose, Sigma), the absolute configuration of the monosaccharide in **2** and **4**−**8** was confirmed to be D-glucose (t_R 12.67 min).

Cytotoxicity Assay. Cell culture and cytotoxic assays against human colon adenocarcinoma (HCT-116), human breast adenocarcinoma (MCF-7), human breast adenocarcinoma (SK-BR-3), human ovarian adenocarcinoma (SK-OV-3), human cervix adenocarcinoma (HeLa), human hepatoma (HepG2), and human melanoma (SK-MEL-5) were performed employing the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich] assay as described in the literature.[16] The reference substance used was

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paclitaxel, which exhibited IC_{50} values of 0.05, 0.90, 0.11, 0.40, and 0.15 uM for the HCT-116, MCF-7, SK-BR-3, SK-OV-3, and SK-MEL-5 cell lines, respectively.

Measurement of NO Production and Cell Viability. This assay was carried out as previously described.[17] Briefly, RAW 264.7 macrophages were harvested and seeded in 96-well plates (1×10^4 cells/well) for measurement of NO production. The plates were pretreated with various concentrations of samples for 30 min and incubated with LPS (1 μg/mL) for 24 h. The amount of NO was determined by the nitrite concentration in the cultured RAW264.7 macrophage supernatants using the Griess reagent. The cell viability was evaluated by MTT reduction. Aminoguanidine (Sigma), a well-known NOS inhibitor, was tested as a positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR, ^{13}C NMR, and HRFABMS spectra of **1**−**8** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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