ent-Kaurane Diterpenoids from *Isodon japonicus*

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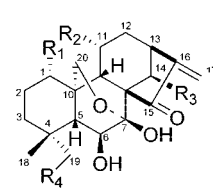
Five *ent*-kaurane diterpenoids, 6*β*,7*β*,14*β*-trihydroxy-1α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one (**1**), 1α,6*β*,7*β*-trihydroxy-11α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one (**2**), 6-hydroxy-1α,19-diacetoxy-6,7-seco-*ent*-kaur-16-en-15-one-7,20-olide (**3**), 19-hydroxy-1α,6-diacetoxy-6,7-seco-*ent*-kaur-16-en-15-one-7,20-olide (**4**), and 6-aldehyde-1α,19-diacetoxy-6,7-seco-*ent*-kaur-16-en-15-one-7,20-olide (**5**), along with 10 known *ent*-kaurane diterpenoids, pseurata C (**6**), longikaurin C (**7**), effusanin C (**8**), longikaurin B (**9**), longikaurin D (**10**), effusanin D (**11**), excisanin B (**12**), lasiokaurin (**13**), megathyrin A (**14**), and loxothyrin A (**15**), were isolated from the aerial parts of *Isodon japonicus*. Their structures were determined on the basis of spectroscopic (1D-, 2D-NMR and MS) and chemical evidence. The isolates were evaluated for their inhibitory effects on LPS-induced production of nitric oxide in murine macrophage RAW264.7 cells.

The genus *Isodon* is a rich source of diterpenoids, and many of these diterpenoids have anti-inflammatory, antitumor, antibacterial, or antifeeding effects.[1] *Isodon japonicus* (Burm.) Hara (Labiatae) is a perennial plant that is widely distributed in Korea, China, and Japan. The aerial parts of this plant have been used in traditional Korean folk medicine to treat gastrointestinal disorders, tumors, and inflammatory diseases.[2,3] L-Arginine-derived nitric oxide (NO) is an intracellular mediator that is produced in mammalian cells by three types of nitric oxide synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). The excessive production of NO by iNOS is important in inflammatory diseases such as rheumatoid arthritis and chronic inflammation. Therefore, inhibition of NO in macrophages might be of therapeutic benefit in inflammatory conditions.[4,5]

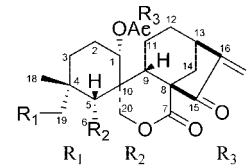
As part of our ongoing research program for the discovery of plant-derived inhibitors of NO production, five new *ent*-kaurane diterpenoids, isodojaponin A−E (**1**−**5**), along with 10 known *ent*-kaurane diterpenoids (**6**−**15**), were isolated from the aerial parts of *I. japonicus*. This paper reports their isolation, structure determination, and inhibition of NO production in murine macrophage RAW264.7 cells.

A MeOH extract of the aerial parts of *I. japonicus* was partitioned by successive extraction with *n*-hexane, CH_2Cl_2, and H_2O. The CH_2Cl_2-soluble fraction was subjected to sequential column chromatography on silica gel, RP-18, and preparative HPLC to afford compounds **1**−**15**.

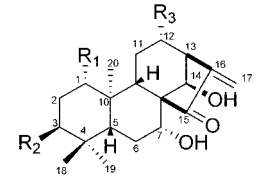
Compound **1** was obtained as a white, amorphous powder having the molecular formula $C_{24}H_{32}O_9$ as determined by HRFABMS, $[M + H]^+$ ion at *m/z* 465.2130 (calcd 465.2125), indicating nine double-bond equivalents in the molecule. A five-membered-ring ketone conjugated with an *exo*-methylene group was evident from the following spectroscopic data: UV λ_{max} at 240.6 nm; IR ν_{max} at 1724 and 1648 cm^{-1}; ^1H NMR δ_H 6.27 and 5.49 (each 1H, s); ^{13}C NMR δ_C 209.2 (s), 152.9 (s), and 119.6 (t).[6,7] The ^1H and ^{13}C NMR spectra also showed signals due to one tertiary methyl group [δ_H 1.41 (3H, s); δ_C 27.0 (q)], two acetoxy groups [δ_H 1.99 (3H, s); δ_C 21.3 (q), 170.9 (s), and δ_H 2.03 (3H, s); δ_C 20.7 (q), 170.1 (s)], six methylene groups [including two oxygenated ones, δ_H 4.80 and 4.44 (each 1H, d, J = 11.5 Hz); δ_C 66.2 and δ_H 4.57 and 4.34 (each 1H, d, J = 10.5 Hz); δ_C 64.0], six methine groups [including three oxygenated ones, δ_H 4.88; δ_C 75.4, δ_H 4.43; δ_C 73.3, and δ_H 5.23; δ_C 73.4], three quaternary carbons (δ_C 37.5, 62.4, and 40.2), and a quaternary hemiacetal carbon (δ_C 98.4). The NMR and MS indicated compound **1** to be a 7*β*-hydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one diterpenoid, with two acetoxy and two additional OH groups. The epoxy hemiacetal linkage between C-7 and C-20 was suggested by the HMBC spectrum of **1**, in which the H-20b signal at δ_H 4.34 was coupled to C-9 at δ_C 52.4 and C-7 at δ_C 98.4, and the H-20a signal at δ_H 4.57 was coupled to C-5 at δ_C 61.3 and C-10 at δ_C 40.2. The acetoxy group was placed at C-1 according to HMBC correlations of H-1 [δ_H 4.88 (1H, dd, J = 11.0, 5.0 Hz)] with C-3 (δ_C 32.9), C-10 (δ_C 40.2), C-20 (δ_C 64.0), and the acetoxy carbonyl carbon (δ_C 170.9). The single tertiary methyl signal at δ_C 27.0 (CH_3-18) and oxygenated methylene signal at δ_C 66.2 (C-19) suggested the presence of another acetoxy group at C-19, which was confirmed by HMBC correlations between CH_2-19 [δ_H 4.80 and 4.44 (each 1H, d, J = 11.5 Hz)] and the ester carbonyl at δ_C 170.1. The two additional OH groups were located at C-6 and C-14 on the basis of HMBC correlations from H-6 (δ_H 4.43) to C-4 (δ_C 37.5), C-7 (δ_C 98.4), and C-8 (δ_C 62.4) and from H-14 (δ_H 5.23) to C-15 (δ_C 209.2) and C-16 (δ_C 152.9). α-Orientation of the acetoxy group at C-1 was indicated by the large coupling constants of H-1*β* with H-2α (J = 11.0 Hz) and H-2*β* (J = 5.0 Hz) and was confirmed by the NOE correlation between H-1 and H-5, which is



	R_1	R_2	R_3	R_4
1	OAc	H	OH	OAc
2	OH	OAc	H	OAc
7	H	H	H	OAc
8	OH	H	H	OAc
9	H	H	OH	OAc
10	H	OH	H	OAc
11	OAc	H	H	OAc
13	OAc	H	OH	H
14	OH	H	OH	H



	R_1	R_2	R_3
3	OAc	CH_2OH	H
4	OH	CH_2OAc	H
5	OAc	CHO	H
15	OAc	CHO	OH



	R_1	R_2	R_3
6	H	OAc	OH
12	OH	H	OAc

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Table 1. NMR Spectroscopic Data (500 MHz, C_5D_5N) for Compounds **1** and **2**[a]

	1		**2**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1β	4.88 dd (11.0, 5.0)	75.4 d[c]	3.75 dd (10.5, 5.5)	73.2 d
2α	1.61 br d (11.0)	25.1 t	1.83[b]	30.3 t
2β	1.88[b]		1.84[b]	
3α	1.84[b]	32.9 t	1.89[b]	34.2 t
3β	1.27[b]		1.25[b]	
4		37.5 s		37.2 s
5β	1.78 br d (7.0)	61.3 d	1.72 d (9.5)	60.0 d
6α	4.43 dd (10.0, 7.0)	73.3 d	4.52 dd (11.5)	74.4 d
7		98.4 s		96.4 s
8		62.4 s		59.3 s
9β	1.97 dd (13.0, 6.0)	52.4 d	1.85[b]	54.1 d
10		40.2 s		43.1 s
11α	2.11 m	18.4 t		70.3 d
11β	1.30[b]		5.73 t (4.5)	
12α	2.36 ddd (11.5, 9.0, 4.0)	30.3 t	2.59 dd (15.5, 9.5)	38.5 t
12β	1.48 m		1.85[b]	
13α	3.19 d (9.5)	43.7 d	3.04 dd (9.5, 4.5)	34.3 d
14α	5.23 s	73.4 d	3.22 d (12.5)	27.3 t
14β			2.55 dd (12.5, 4.5)	
15		209.2 s		209.2 s
16		152.9 s		153.3 s
17a	6.27 s	119.6 t	6.04 s	118.1 t
17b	5.49 s		5.35 s	
18	1.41 s	27.0 q	1.49 s	29.1 q
19a	4.80 d (11.5)	66.2 t	4.78 d (11.0)	67.4 t
19b	4.44 d (11.5)		4.45 d (11.0)	
20a	4.57 d (10.5)	64.0 t	5.08 d (9.5)	65.5 t
20b	4.34 d (10.5)		4.34 d (9.5)	
OAc-1 or	1.99 s	21.3 q	2.09 s	22.1 q
OAc-11		170.9 s		170.2 s
OAc-19	2.03 s	20.7 q	1.94 s	20.8 q
		170.1 s		170.8 s
OH-6	7.09 d (10.5)		6.41, 1H, d, (11.5)	

[a] The assignments were based on the DEPT, HMQC, and HMBC experiments. [b] The multiplicity patterns were unclear due to signal overlapping. [c] Carbon multiplicity.

β-oriented in *ent*-kaurane-type diterpenes.[8] The OH groups at C-6 and C-14 were both β-orientated according to the NOESY correlations of H-6α (δ_H 4.43, dd, J = 10.0, 7.0 Hz) with H-19a (δ_H 4.80, d, J = 11.5 Hz) and H-20b (δ_H 4.34, d, J = 10.5 Hz) and H-14α (δ_H 5.23, s) with H-11α (δ_H 2.11, m) and H-20a (δ_H 4.57, d, J = 10.5 Hz). A computer-modeled 3D structure of **1** obtained using the molecular modeling program, with MM2 force-field calculations for energy minimization, was in good agreement with the observed NOESY correlations. Consequently, compound **1** was determined to be 6β,7β,14β-trihydroxy-1α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one, and it was named isodojaponin A.

Compound **2** had a molecular formula determined to be $C_{24}H_{32}O_9$ by positive HRFABMS. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of enanderianin A (1β,6β,7β-trihydroxy-11α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one)[6] showed the only difference to be the configuration at C-1. α-Orientation of the OH group at C-1 was confirmed from the chemical shift and coupling pattern of H-1β (δ_H 3.75, dd, J = 10.5, 5.5 Hz), which was supported by the downfield shift of C-11 (δ_C 70.3) caused by the δ-*syn*-axial effect between the 1α-OH group and C-11.[9] NOESY correlations of H-1β (δ_H 3.75) with H-5β (δ_H 1.72), H-9β (δ_H 1.85), and H-11β (δ_H 5.73) supported the α-orientation of OH-1. Therefore, compound **2** was determined to be 1α,6β,7β-trihydroxy-11α,19-diacetoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one, and it was named isodojaponin B.

Compound **3** exhibited a peak at *m*/*z* 449.2207 ([M + H]$^+$, calcd 449.2170) (HRESIMS), in agreement with the molecular formula $C_{24}H_{32}O_8$ and indicating nine degrees of unsaturation. The ^{1}H NMR spectrum (Table 2) showed singlets at δ_H 5.95 (1H, s) and 5.34 (1H, s) that were assigned to an *exo*-methylene, three pairs of AB doublets δ_H 4.08, 3.94 (each 1H, d, J = 12.1 Hz), 4.34, 4.25 (each 1H, d, J = 11.4 Hz), and 5.17, 5.06 (each 1H, d, J = 12.2 Hz) that were assigned to an oxygenated methylene, a signal at δ_H 5.10 (1H, d, J = 9.1 Hz) that was assigned to oxygen-bearing methines, signals at δ_H 1.97 (3H, s) and 2.17 (3H, s) that were assigned to acetoxyl groups, and a signal at δ_H 1.13 (3H, s) due to a tertiary methyl group. The ^{13}C NMR and DEPT spectra showed **3** to contain 24 carbons, including a conjugated ketone (δ_C 202.5), an *exo*-methylene (δ_C 118.4 and 151.4), a δ lactone (δ_C 170.8), three oxygenated methylenes (δ_C 58.0, 67.4, and 69.3), a methine (δ_C 76.8) bearing an acetoxy group, two acetyl groups (δ_C 20.7, 21.5, 170.3, and 170.6), and a methyl group (δ_C 28.1). On the basis of the characteristic lactone carbonyl signal at δ_C 170.8 due to C-7 and oxygenated methylene signals [δ_C 69.3, C-20; δ_H 5.17 and 5.06 (d, J = 12.2 Hz), H-20 which showed HMBC correlations with C-1, C-7, and C-10], compound **3** was presumed to have a 6,7-seco-7,20-olide *ent*-kauranoid skeleton, with an OH and two acetoxy groups.[10–12] According to the cross-peaks in the HMBC spectrum of compound **3**, an OH and two acetoxy groups were placed at C-6, C-1, and C-19, respectively. These substituents were α-orientated, as indicated by NOESY correlations of δ_H 5.10 (H-1β) with δ_H 1.91 (H-5β) and 1.35 (H-3β); δ_H 4.34 and 4.25 (H-19) with δ_H 2.08 (H-2α) and 5.06 (H-20b); and δ_H 4.08 and 3.94 (H-6) with δ_H 5.17 (H-20a) and 4.34 (H-19a). Thus, compound **3** was identified as 6-hydroxy-1α,19-diacetoxy-6,7-seco-*ent*-kaur-16-en-15-one-7,20-olide, and it was named isodojaponin C.

Compound **4** had the molecular formula $C_{24}H_{32}O_8$ (HRESIMS). The MS and NMR data were similar to those of compound **3** and to rabdokaurin B.[12] Comparison of the ^{1}H and ^{13}C NMR spectra of **4** with those of **3** showed the only difference to be the positions of acetoxy and OH groups. The chemical shift differences of the signals for C-5 [δ_C 50.8 ($\Delta\delta$ −2.9 ppm)], C-6 [δ_C 62.4 ($\Delta\delta$ +4.4 ppm)], C-18 [δ_C 29.5 ($\Delta\delta$ +1.4 ppm)], H-6 [δ_H 5.01 ($\Delta\delta$ −0.93 ppm) and 4.42 ($\Delta\delta$ −0.48 ppm)], and H-19 [δ_H 3.84 ($\Delta\delta$ +0.5

Table 2. NMR Spectroscopic Data (500 MHz, C_5D_5N) for Compounds **3**−**5**[a]

	3		**4**		**5**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1β	5.10 d (9.1)	76.8 d[c]	5.07 dd (12.0, 3.0)	76.9 d	5.07 dd (11.5, 3.5)	75.3 d
2α	2.08 br d (14.0)	24.6 t	2.07 m	24.6 t	2.15 m	24.0 t
2β	1.85 m		1.92 dd (12.0, 3.5)		1.19 m	
3α	1.81 br d (14.0)	34.5 t	1.65 m	35.2 t	1.67 m	34.8 t
	1.35 dd (14.0, 3.6)		1.37 m		1.51 m	
4		38.2 s		39.6 s		39.1 s
5β	1.91[b]	53.7 d	1.96[b]	50.8 d	2.77 d (3.5)	60.9 d
6	4.08 d (12.1)	58.0 t	5.01 d (13.5)	62.4 t	10.10 d (3.5)	201.1 d
	3.94 d (12.1)		4.42 dd (13.5, 7.0)			
7		170.8 s		170.2 s		170.3 s
8		58.6 s		58.0 s		58.6 s
9β	3.18 dd (13.1, 3.8)	42.4 d	2.68 dd (13.0, 4.0)	43.0 d	2.62 dd (13.5, 4.5)	43.1 d
10		44.6 s		44.3 s		43.6 s
11α		17.9 t	1.53 m	17.9 t	1.55 m	17.4 t
11β	1.58 m		1.62 m		1.58 m	
12α	2.00 m	30.1 t	2.11 m	29.8 t	2.09 m	30.6 t
12β	1.43 m		1.42 m		2.00 m	
13α	2.92 dd (9.1, 4.5)	35.3 d	2.97 dd (9.0, 4.5)	35.2 d	2.89 dd (9.0, 4.5)	35.0 d
14α	2.16 m	29.3 t	2.18 br d (12.5)	29.0 t	2.13 m	29.1 t
14β	2.58 dd (12.3, 4.5)		2.61 dd (12.5, 4.5)		2.55 dd (12.5, 4.5)	
15		202.5 s		202.1 s		201.9 s
16		151.4 s		151.4 s		150.8 s
17	5.95 s	118.4 t	6.08 s	118.8 t	6.00 s	119.1 t
	5.34 s		5.47 s		5.36 s	
18	1.13 s (3H)	28.1 q	1.26 s (3H)	29.5 q	1.21 s (3H)	28.1 q
19	4.34 d (11.4)	67.4 t	3.84 d (11.5)	67.6 t	4.55 d (12.0)	68.8 t
	4.25 d (11.4)		3.62 d (11.5)		4.21 d (12.0)	
20	5.17 d (12.2)	69.3 t	5.43 d (12.5)	68.8 t	5.42 br s (2H)	67.6 t
	5.06 d (12.2)		4.92 d (12.5)			
OAc-1	2.17 s (3H)	170.6 s	2.19 s (3H)	169.9 s	2.16 s (3H)	170.3 s
		20.7 q		20.9 q		20.4 q
OAc-6 or	1.97 s (3H)	170.3 s	1.97 s (3H)	170.6 s	1.93 s (3H)	170.2 s
OAc-19		21.5 q		21.4 q		21.3 q

[a] The assignments were based on the DEPT, HMQC, and HMBC experiments. [b] The multiplicity patterns were unclear due to signal overlapping. [c] Carbon multiplicity.

ppm) and 3.62 ($\Delta\delta$ +0.63 ppm)] in compound **4** were observed. HMBC correlations from H-6 (δ_H 5.01 and 4.42) to an acetoxy carbonyl carbon (δ_C 170.6) and from H-1 (δ_H 5.07) to an acetoxy carbonyl carbon (δ_C 169.9) clearly indicated the acetoxy group to be at C-6 in **4** rather than at C-19 as in **3**. Cross-peaks in the NOESY spectrum of **4** indicated that the corresponding substituents in compound **4** had the same orientations as those in **3**. Therefore, compound **4** was identified as 19-hydroxy-1α,6-diacetoxy-6,7-seco-*ent*-kaur-16-en-15-one-7,20-olide, and it was named isodojaponin D.

The molecular formula of compound **5** was $C_{24}H_{30}O_8$ (HRES-IMS), and the ^{1}H and ^{13}C NMR and DEPT spectra of **5** were similar to those of loxothyrin A (**15**),[13] except for the absence of an OH group at C-11. Acetoxy groups were placed at C-1 and C-19 by the HMBC correlations of H-1 (δ_H 5.07) and H-19 (δ_H 4.55 and 4.21) with an acetoxy carbonyl carbon at δ_C 170.2 and 170.3, respectively. An aldehyde group was indicated at C-6 by the HMBC interaction of H-6 (δ_H 10.10) and C-4 (δ_C 39.1), C-5 (δ_C 60.9), and C-10 (δ_C 43.6). The relative configuration of compound **5** was the same as that of **4** on the basis of their similar NOESY spectra. Thus, compound **5** was identified as 6-aldehyde-1α,19-diacetoxy-6,7-seco-*ent*-kaur-16-en-15-one-7,20-olide and was called isodojaponin E.

Ten known diterpenoids were identified by comparing their spectroscopic data with those reported in the literature: pseurata C (**6**),[14] longikaurin C (**7**),[15] effusanin C (**8**),[16] longikaurin B (**9**),[10] longikaurin D (**10**),[17] effusanin D (**11**),[16] excisanin B (**12**),[18] lasiokaurin (**13**),[11] megathyrin A (**14**),[19] and loxothyrin A (**15**).[13]

All isolates (**1**−**15**) were tested for inhibition of LPS-induced NO production in RAW264.7 cells, with aminoguanidine as the positive control. As shown in Table 3, all compounds inhibited NO production, with IC_{50} values ranging from 0.9 to 24.2 μM. None of the compounds had any significant cytotoxicity in the CCK assay at concentrations where they inhibited NO production (IC_{50} value for compounds **1**−**4** and **6**−**14**: >20 μM; compounds **5** and **15**: >40 μM). All compounds possess an α-methylenecyclopentanone group, which may represent the active moiety.[1,20]

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-1000 polarimeter. UV and IR spectra were obtained on a JASCO UV-550 and on a Perkin-Elmer model LE599 spectrometer, respectively. The ^{1}H, ^{13}C, and 2D-NMR spectra were recorded on a Bruker DRX 500 spectrometer using C_5D_5N as solvent. HR-FABMS and EIMS spectra were obtained on JMS 700 (JEOL, Tokyo, Japan) and VG Autospec Ultima (Micromass, Manchester, UK) mass spectrometers, respectively. Preparative HPLC was carried out on a Waters system (two 515 pumps and a 2996 photodiode array detector) and a YMC J'sphere ODS-H80 column (4 μm, 150 × 20 mm), using the mixed solvent system CH_3CN-H_2O at a flow rate of 6.0 mL/min. Open-column chromatography was performed using silica gel (Kieselgel 60, 70−230 mesh, Merck) and Lichroprep RP-18 (40−63 μM, Merck), and thin-layer chromatography (TLC) was performed using precoated silica gel 60 F_{254} (0.25 mm, Merck).

Plant Material. The aerial parts of *I. japonicus* were collected from Hwacheon, Kangwondo, Korea, in September 2003. The plant material was identified by Emeritus Professor Kyong Soon Lee, who is a plant taxonomist at Chungbuk National University. A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Chungbuk National University, Korea (CBNU0309).

Extraction and Isolation. The air-dried aerial parts of *I. japonicus* (1.7 kg) were pulverized and extracted with MeOH (3 × 15 L) at room temperature (24 h). The extract was filtered and concentrated *in vacuo*, diluted with H_2O, and then partitioned with *n*-hexane (3 × 1.5 L) and CH_2Cl_2 (3 × 1.5 L). The CH_2Cl_2-soluble extract inhibited 65% of NO production in LPS-stimulated RAW264.7 cells at a concentration of 3 μg/mL. The CH_2Cl_2-soluble extract (17.5 g) was then subjected to column chromatography (CC) on silica gel eluted with CH_2Cl_2-MeOH

Table 3. Inhibition of NO Production by Compounds **1**−**15**[a]

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	6.3 ± 0.22	**9**	0.9 ± 0.08
2	10.2 ± 0.25	**10**	2.0 ± 0.11
3	8.2 ± 0.12	**11**	3.3 ± 0.11
4	8.7 ± 0.27	**12**	3.3 ± 0.05
5	20.3 ± 0.33	**13**	1.6 ± 0.13
6	5.4 ± 0.09	**14**	8.5 ± 0.15
7	1.0 ± 0.15	**15**	24.2 ± 0.25
8	5.9 ± 0.06	AG[b]	32.2 ± 0.07

[a] Data are presented as a mean ± SD from three separate experiments. [b] Aminoguanidine was used as the positive control.

(100:0 to 1:1, then pure MeOH), to yield five fractions (IJA−IJE). Fractions IJB, IJC, and IJD inhibited 82%, 89%, and 75% of NO production at 3 μg/mL, respectively. Fraction IJB (5.1 g) was subjected to CC over silica gel eluted with *n*-hexane−acetone (5:1, 3:1, 3:2, 1:1, 1:2) to yield fractions IJB-1−IJB-6. Fraction IJB-4 (621 mg) was subjected to flash CC on RP-18 (40−63 μm) eluted with CH_3CN-H_2O (30:70) to afford fractions IJB-41−IJB-43. Fraction IJB-42 was further purified by preparative HPLC eluted with CH_3CN-H_2O (50:50) to yield compounds **6** (3.2 mg) and **11** (21.3 mg). Compound **7** (6.5 mg) was obtained from fraction IJB-43 through preparative HPLC eluted with CH_3CN-H_2O (50:50). Fraction IJB-6 was purified over a silica gel column (CH_2Cl_2−acetone, 100:0 to1:1), then by preparative HPLC with CH_3CN-H_2O (45:55), to yield **1** (14.5 mg). Fraction IJB-5 was subjected to RP-18 (40−63 μm) eluted with CH_3CN-H_2O (10:90 to 30:70) to give fractions IJB-51−IJB-54. Compounds **12** (11.1 mg) and **13** (5.6 mg) were isolated from fraction IJB-53 by silica gel CC with a gradient elution of CH_2Cl_2−acetone (100:0 to1:1), followed by preparative HPLC eluted with CH_3CN-H_2O (42:58). Compounds **3** (15.4 mg), **4** (14.2 mg), and **15** (14.9 mg) were isolated from IJB-53 using a silica gel column with gradient elution (CH_2Cl_2−acetone, 100:0 to 1:1), followed by semipreparative HPLC eluted with CH_3CN-H_2O (42:58). Fraction IJB-54 was separated directly by semipreparative RP-HPLC eluting with CH_3CN-H_2O (42:58) to yield **5** (2.4 mg). Fraction IJC (2.9 g) was applied to CC on silica gel eluted with *n*-hexane−acetone (5:1, 3:1, 3:2, 0:1) to afford fractions IJC-1−IJC-7. Fraction IJC-5 (968 mg) was subjected to flash CC on RP-18 (40−63 μm) eluted with CH_3CN-H_2O (10:90 to 30:70) to give fractions IJC-51−IJC-55. Compound **8** (134.4 mg) was obtained from fraction IJC-52 by preparative HPLC eluted with CH_3CN-H_2O (35:65). Fraction IJC-51 was separated by preparative HPLC eluted with CH_3CN-H_2O (30:70 to 50:50) to yield **2** (3.5 mg). Compounds **9** (14.6 mg) and **10** (5.8 mg) were isolated from fraction IJC-54 by preparative HPLC eluted with CH_3CN-H_2O (40:60). Fraction IJD (4.1 g) was subjected to vacuum liquid chromatography on RP-18 eluted with CH_3CN-H_2O (20%, 40%, 60%, 80%, and 100%) to give fractions IJD-1−IJD-6. Fraction IJD-4 was purified by preparative HPLC with CH_3CN-H_2O (30:70) to yield compound **14** (11.5 mg).

Isodojaponin A (1): white, amorphous powder; $[\alpha]^{25}_D$ −51 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 240.6 (3.95); IR (KBr) ν_{max} 3428, 1724, 1648, 1442, 1345 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; EIMS *m*/*z* 464 $[M]^+$ (39), 446 (14), 404 (92), 386 (17), 358 (10), 344 (84), 298 (43), 223 (33), 147 (21), 91 (100); HRFABMS *m*/*z* 465.2130 $[M + H]^+$ (calcd for $C_{24}H_{33}O_9$, 465.2125).

Isodojaponin B (2): white, amorphous powder; $[\alpha]^{25}_D$ −32 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 238.2 (3.99); IR (KBr) ν_{max} 3435, 1733, 1642, 1443, 1350, 1028 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz), see Table 1; EIMS *m*/*z* 464 $[M]^+$ (19), 404 (100), 386 (13), 344 (45), 326 (37), 313 (26), 298 (18), 280 (20); HRFABMS *m*/*z* 465.2123 $[M + H]^+$ (calcd for $C_{24}H_{33}O_9$, 465.2125).

Isodojaponin C (3): colorless needles; $[\alpha]^{25}_D$ +22.9 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 235.9 (3.95) nm; IR (KBr) ν_{max} 3431, 2925, 1725, 1710, 1637, 1432, 1355 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; EIMS *m*/*z* 448 $[M]^+$, 406 $[M - OAc]^+$, 388, 360, 346, 328, 310, 300, 253, 149, 133, 119; HRESIMS *m*/*z* 449.2207 (calcd for $C_{24}H_{32}O_8H$ 449.2170).

Isodojaponin D (4): white, amorphous powder; $[\alpha]^{25}_D$ +38.6 (*c* 0.03, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 234.7 (3.76) nm; IR (KBr) ν_{max} 3445, 1737, 1705, 1648, 1432, 1350 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; EIMS *m*/*z* 448 $[M]^+$, 406, 388, 360, 346, 328, 310, 300, 253, 149, 133, 119; HRESIMS *m*/*z* 449.2199 (calcd for $C_{24}H_{32}O_8H$ 449.2170).

Isodojaponin E (5): white, amorphous powder; $[\alpha]^{25}_D$ +13.3 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 234.7 (3.94) nm; IR (KBr) ν_{max} 1735, 1708, 1642, 1425, 1338, 1025 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; EIMS *m*/*z* 446 $[M]^+$, 418, 376, 358, 344, 326, 298, 119, 105; HRESIMS *m*/*z* 464.2267 (calcd for $C_{24}H_{30}O_8NH_4$ 464.2278).

Determination of NO Production and Cell Viability. The level of nitric oxide production was determined by measuring the amount of nitrite in the cell culture supernatant as previously described.[15] Briefly, RAW264.7 cells (2×10^5 cells/well) were stimulated with or without 1 μg/mL LPS for 24 h (Sigma Chemical Co., St. Louis, MO) in the presence or absence of test compounds. The cell culture supernatant (100 μL) was reacted with 100 μL of Griess reagent. The viability of the cells remaining after the Griess assay was determined using a CCK-8 assay (Cell Counting Kit-8, Dojindo, Tokyo, Japan).

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