Labdane Diterpenoid Glycosides from *Alpinia densespicata* and Their Nitric Oxide Inhibitory Activities in Macrophages

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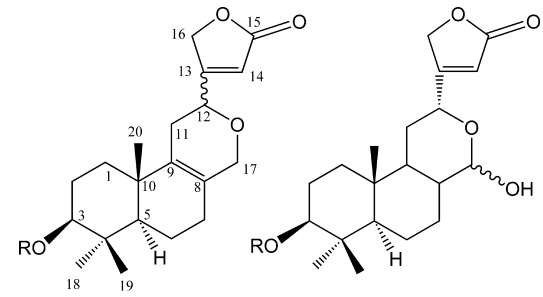
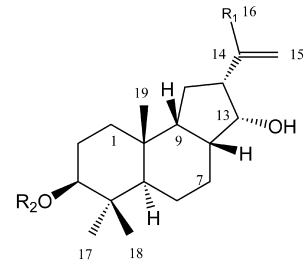
Four new labdane diterpene glycosides (**1**−**4**) named alpindenosides A, B, C, and D, two new norditerpene glycosides (**5**, **6**) named noralpindenosides A and B, and three known flavonoid glycosides (**7**−**9**) have been isolated from the stems of *Alpinia densespicata*. Structural elucidation of compounds **1**−**6** was based on spectroscopic data. Compounds **1**−**9** all exhibited moderate NO inhibitory activities, whereas they were noncytotoxic at 20 μM against several human tumor cell lines.

The plant family Zingiberaceae has about 1400 species, and most of them grow or are cultivated in tropical and subtropical Asia. The plants typically have large rhizomes that are used for food, spice, or traditional medicines. Plants of this family have been reported to possess antioxidant,[1,2] anti-inflammatory,[2] cytotoxic,[3] and hepatoprotective activities.[4] Some *Alpinia* species have been used as Chinese herbal drugs for antiemetic and stomachic purposes.[5] *Alpinia* species have also been reported to have antitumor, pungency, antibacterial, antiulcer, antifungal, and insecticidal properties.[6−8] We report herein the separation of an EtOH extract of *Alpina densespicata* Hayata (Zingiberaceae), by column chromatography and HPLC, to yield new labdane diterpene and norditerpene lactone glycosides (**1**−**6**), along with the known flavonoid glycosides kaempferol-3-*O*-α-L-rhamnosyl-(1→2)-*O*-L-rhamnoside (**7**), quercetin-3-*O*-α-L-rhamnosyl-(1→2)-*O*-α-L-rhamnoside (**8**), and morin-7-*O*-*β*-D-glucopyranoside (**9**). Structural elucidation of compounds **1**−**6** was based on spectroscopic analysis, mainly using HRESIMS, IR, and 1D and 2D NMR experiments. Compounds **1**−**9** were then evaluated for anti-inflammatory and antioxidant activities and inhibition of human tumor cell lines.



	R
1	-Glc$_2$-Rha
2	-Glc$_2$-Rha
1a	H
2a	H

	R
3	-Glc$_2$-Rha
4	-Glc$_2^6$(OAc)-Rha



	R_1	R_2
5	-CH_2OH	-Glc$_2$-Rha
6	-CH_3	-Glc$_2$-Rha

Results and Discussion

Compound **1** was obtained as a white powder and was determined to have the molecular formula $C_{32}H_{48}O_{13}$ on the basis of HRESIMS; $[M + Na]^+$ at *m*/*z* 663.3038. The IR spectrum of **1** showed absorption bands at 1746 and 1656 cm^{-1} ascribable to lactone ring and C=C functions, respectively, and strong absorption bands at 3387 and 1042 cm^{-1} suggestive of an oligoglycoside.[9] Two anomeric carbon signals (δ 105.6, 101.8) and signals characteristic for three methyl groups and one oxygenated methylene group were present in the ^{1}H and ^{13}C NMR and DEPT spectra (Table 1). Other functional groups including a lactone ring were identified by the presence of characteristic resonances at δ 175.9 (C-15), 73.94 (C-16), and 135.0, 149.0 (C-13, -14), and the HMBC spectrum showed that H-16 was correlated with carbonyl (C-15), olefinic (C-14), and oxygenated (C-12) carbons. Thus, the lactone ring was linked at C-12.

The ^{1}H−^{1}H COSY spectrum of **1** showed cross-peaks between H-1, H-2, and H-3, between H-5, H-6, and H-7, and between H-11 and H-12. The HMBC spectrum (Figure 1) showed correlations between two protons (H-12, H-17) and olefinic quaternary carbons (C-8, C-9) and between proton H-17 and secondary carbon C-7, suggesting that two six-membered rings were fused to a six-membered cyclic ether moiety. In addition, long-range correlations occurred between H-12 and olefinic carbons C-9 and C-13, as well as between three singlet methyl groups and quaternary carbons C-4 and C-10. These data suggested that **1** was a substituted labdane-type diterpene.[10]

The two sugar moieties were identified as glucose (Glc) and rhamnose (Rha) on the basis of characteristic ^{13}C NMR data, δ 105.6, 79.4, 78.9, 77.6, 72.0, 62.9 for glucose and δ 101.8, 74.0, 72.1, 72.1, 70.0, 18.0 for rhamnose.[11] In the ^{1}H NMR spectrum, anomeric protons at δ 4.42 (d, 7.6) and 5.37 (s) and a typical rhamnose methyl signal (δ 1.21, d, 6.0) were observed. The glucose

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Table 1. ^{1}H NMR Data (δ, ppm) of **1**−**6** (MeOD, 600 and 500 MHz)

no.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]
1	1.32, td (11.4, 3.6)	1.20, m	1.16, dt (14.0, 3.0)	1.10, m	1.10, m	1.10, m
	1.71, d (12.0)	1.66, d (12.5)	1.62, dd (13.0,3.0)	1.64, m	1.45, m	1.45, m
2	1.72, d (13.2)	1.67, d (12.5)	1.64, m	1.65, m	1.66, m	1.66, m
	2.08, (dd, 13.2, 3.2)	2.04, dd (14.0, 3.5)	2.01, m	1.93, m	1.97, dd (13.0, 4.0)	1.97 dd (13.0, 4.0)
3	3.20, dd (12.0, 4.2)	3.18, dd (12.0, 4.0)	3.25, dd (11.5, 4.0)	3.14, dd (11.5, 3.5)	3.17, dd (11.5, 4.0)	3.17 dd (11.5, 4.0)
5	1.24, m	1.15, d (12.0)	0.94, dd (13.5, 1.5)	0.94, d (12.0)	0.84, m	0.85, m
6	1.77, dd (12.6, 6.6)	1.79, td (6.0, 13.5)	1.67, m	1.67, m	1.67, m	1.67, m
	1.58, ddd (19.2, 12.0, 6.0)	55, m	1.38, td (13.0, 4.0)	1.37, td (4.0, 13.0)	1.30, m	1.30, m
7	1.90, d (7.2)	1.92, brd (6.0)	2.07, m	2.05, m	2.09, dd (12.4, 2.8)	2.09, dd (12.4, 2.4)
			0.99, m	0.99, m	0.98, m	0.97, dd (12.5, 4.0)
8			1.31, m (β)	1.31, m (β)	1.43, m (β)	1.43, m (β)
9			1.11, d (10.0, β)	1.10, m (β)	1.12, d (9.5, β)	1.06, m (β)
11	2.42, brd (15.6, α)	2.31, brd, (16.0, β)	91, brd (9.0, β)	1.80, brd (9.0, β)	1.75, dd, (2.5, 12.0, β)	1.66, m (β)
	2.03, m (β)	1.96, m (α)	1.09, m, (α)	1.09, m (α)	1.41, m (α)	1.36, m (α)
12	4.33, brd (7.2,α)	4.15, brd (10.5, β)	4.23, brd (9.0,β)	4.23, d (8.0, β)	2.34, m (β)	2.22, m (β)
13					3.46, t (9.0, β)	3 0.42, m (β)
14	7.54, s	7.54, s	7.50, s	7.56, s		
15					4.93, s	4.84, s
					5.04, s	4.74, s
16	4.88, s	4.88, s	4.90, s	4.85, m	4.05, s (2H)	1.72, s
17	4.06, d (16.2, α)	4.06, d (15.0, α)	4.34, d (8.0, β)	4.35, d (8.0, β)	1.04, s (α)	1.04, s (α)
	3.93, m (β)	3.98, m (β)				
18	1.08, s (α)	1.08, s (α)	1.08, s (α)	1.05, s (α)	0.87, s (β)	0.87, s (β)
19	1.02, s (β)	1.02, s (β)	0.87, s (β)	0.87 s (β)	0.86, s (β)	0.86, s (β)
20	0.90, s (β)	0.90, s (β)	0.85, s (β)	0.87, s (β)		
G1	4.42, d (7.8)	4.42, d (7.5)	4.41, d (7.5)	4.41, d (7.2)	4.40, d (7.6)	4.40, d (7.6)
G2	3.42, d (8.0)	3.42, d (7.0)	3.41, d (7.5)	3.41, d (7.6)	3.42, m	3.43, m
G3	3.45, t (8.5)	3.45, t (8.5)	3.45, t (8.0)	3.45, d (8.4)	3.46, m	3.45, m
G4	3.27, m	3.30, m	3.27, m	3.25, t (8.8)	3.25, m	3.24, m
G5	3.22, m	3.27, m	3.26, m	3.28, m	3.24, m	3.24, d (2.0)
G6	3.64, dd (12, 5.5)	3.66, dd (12, 5.5)	3.64, dd (12, 5.5)	4.22, dd (9.6, 4.4)	3.67, m	3.66, dd (11.6, 5.2)
	3.84, d (11.5)	3.83, d (11.5)	3.84, d (11.5)	4.36, m	3.83, dd (12.0, 2.0)	3.85, dd (11.6, 2.0)
R1	5.37, s	5.36, s	5.36, s	5.37, s	5.36, s	5.36, s
R2	3.95, m	3.92, d (3.2)	3.94, m	3.72, d (3.2)	3.92, m	3.92, d (3.2)
R3	3.75, dd (9.0, 3.0)	3.75, dd (9.0, 3.0)	3.75, dd (9.0, 3.0)	3.75, d (3.2)	3.75, d (3.6)	3.75, d (3.2)
R4	3.40, t (9.2)	3.39, m	3.39, m	3.39, d (9.6)	3.38, m	3.38, m
R5	3.98, m	3.95, m	3. 97, m	3.96, m	3.97, m	3.95, m
R6	1.21, d (6.0)	1.22, d (6.4)	1.20, d (6.0)	1.20, d (6.0)	1.21, d (6.0)	1.21, d (6.0)

[a] 600 MHz. [b] 500 MHz. G = glucose, R = rhamnose.

had a β-configuration and rhamnose had an α-configuration on the basis of the magnitude of the coupling constants of the anomeric protons, J = 7.6 and 0 Hz, respectively. Due to the HMBC cross-peaks of Rha-H-1 with Glc-C-2, and of Glc-H-1 with C-3 of the diterpene, their positions were assigned as Rha-(1→2)-*O*-β-Glc connecting with C-3 of the aglycone. Major cross-peaks of H-3α/Me-18, H-1α, H-5α, H-1α/H-11α, H-11α/H-12α, Me-20/H-1β, and Me-19/H-1β, H-11β were observed in the NOESY spectrum of **1** (Figure 2). Thus, the structure and relative configuration of **1** was assigned to be as indicated, and it was named alpindenoside A.

The molecular formula of **2** (alpindenoside B) was assigned as $C_{32}H_{48}O_{13}$ by HRESIMS (quasimolecular ion peak $[M + Na]^+$ at *m/z* 663.3048). The IR spectrum of **2** was essentially identical to that of **1**. The chemical shift values of C-l to C-20 and the signals of glucose and a rhamnose in the ^{1}H NMR and ^{13}C NMR spectra of **2** were also nearly identical to those of **1**, except for the signals of H-11 and H-12. There were obvious changes of H-11 and H-12 signals between **1** and **2** in their ^{1}H NMR spectra (Table 1). These data, together with NOESY cross-peaks of H-3α/H-1α, H-5α, Me-18, H-1β/H-11β, H-1α/H-11α, and H-11β/H-12β, Me-20, revealed that **2** was the H-12 β-epimer of **1**. Comparing ^{13}C NMR spectra of **1** and **2**, the signal of C-12 for **2** shifted to lower field and was consistent with the structure and relative configuration indicated for **2**. Acid hydrolysis of **1** and **2** with 2 N HCl afforded aglycones **1a** and **2a**, respectively, along with D-glucose and L-rhamnose in a ratio of 1:1, both of which were identified by chiral GC.

Compound **3** was obtained as a white powder, and its IR spectrum with absorption bands at 1743, 3380, and 1046 cm^{-1} indicated lactone ring and oligoglycosidic moieties. The quisamolecular ion $[M + Na]^+$ at *m/z* 681.3195 (HRESIMS) indicated that the formula of **3** was $C_{32}H_{50}O_{14}$. These data, together with signals consistent with three tertiary methyl, one dioxymethine, two glycosidic, and one α,β-unsaturated-γ-lactone group in the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), revealed that **3** was a labdane-type diterpene attached to a glucose and a rhamnose. The ^{1}H NMR and ^{13}C NMR data of **3** were very similar to those of curcumanggoside, except for the presence of the rhamnose signals, and the signal of glucose C-2 in **3** was shifted downfield to δ 79.0.[12] Furthermore, long-range correlation between H-1 of rhamnose and C-2 of glucose was observed in the HMBC spectrum. These findings suggested that the rhamnose was linked to C-2 of the glucose. The relative configuration of C-12 was determined from the NOESY spectrum of **3**, which showed cross-peaks of H-11 (2.31, brd, J = 16.0 Hz)/Me-20, H-12. Thus, H-11 (2.31, brd, J = 16.0 Hz), H-12 should have a β-orientation, because Me-20 is β-oriented. Thus, the structure of **3** was elucidated, and it was named alpindenoside C.

Compounds **4** (alpindenoside D) and **3** had similar IR and ^{1}H and ^{13}C NMR spectra (Table 2), revealing that **4** was a labdane diterpene containing glucose and rhamnose. The HRESIMS spectrum of **4** showed the quasimolecular ion $[M + Na]^+$ at *m/z* 723.3280, indicating a molecular formula of $C_{34}H_{52}O_{15}$. Thus, **4** differed from **3** by an additional C_2H_2O unit. The ^{13}C NMR spectrum of **4** showed signals of an OAc group (δ 172.7, 20.8), and the signal of C-6 of glucose in **3** (δ 62.8) was shifted to lower field in **4** (δ 64.8), suggesting that the OAc group in **4** was linked to C-6 of Glc. Like **3**, H-12 of **4** was β-oriented mainly on the

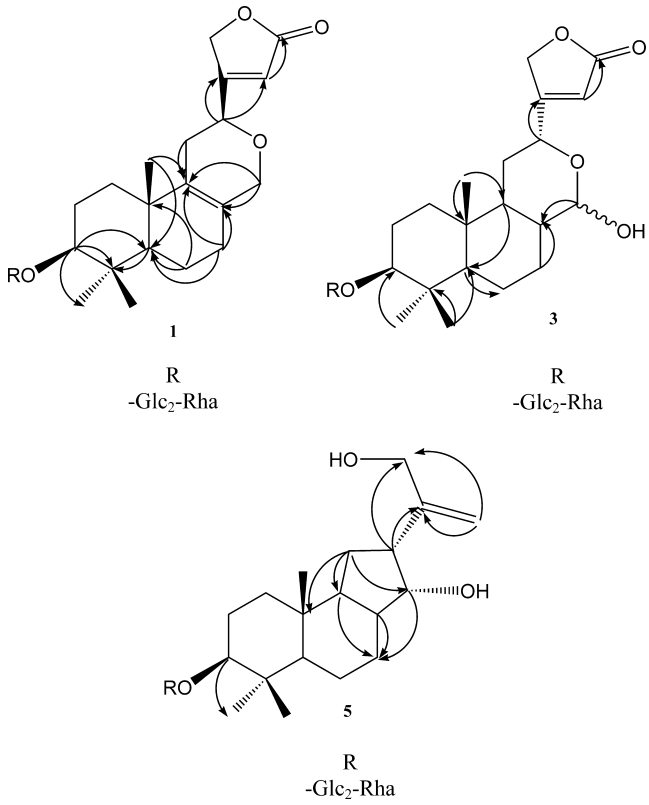

Figure 1. Major HMBC correlations of compounds **1**, **3**, and **5**.

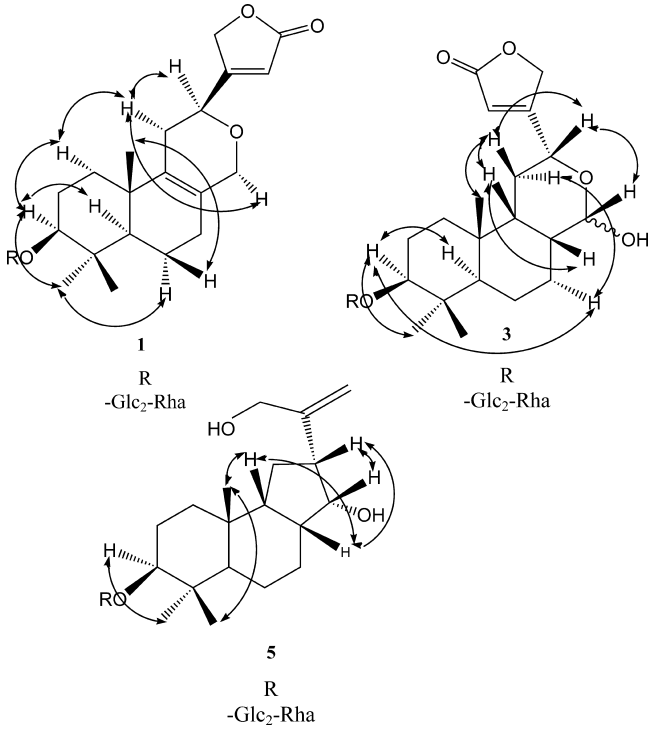

Figure 2. Major NOESY connectivities of compounds **1**, **3**, and **5**.

basis of the characteristic NOE correlations and carbon chemical shift of C-12 (δ 71.4). Thus, the structure of **4** was determined to be as shown.

Isolated labdane diterpenes with a six-membered cyclic ether moiety (such as **1**−**4**) are rarely reported, except for curcumanggoside, which was isolated from another zingiberaceous plant.[12] A postulated biogenetic pathway to **1**−**4** is shown in Scheme 1 (Supporting Information).

Table 2. ^{13}C NMR Data (δ, ppm) of **1**−**6** (MeOD, 100 MHz)

no.[a]	1	2	3	4	5	6
1	36.6, t	35.3, t	38.4, t	38.4, t	38.5, t	38.5, t
2	27.7, t	27.5, t	27.5, t	27.4, t	37.5, t	27.5, t
3	89.9, d	90.1, d	90.1, d	90.4, d	90.4, d	90.4, d
4	40.4, s	40.2, s	40.3, s	40.4, s	40.3, s	40.3, s
5	52.8, d	52.8, d	56.0, d	56.0, d	56.9, d	56.9, d
6	18.9, t	19.0, t	21.4, t	21.7, t	22.3, t	22.4, t
7	28.2, t	27.8, t	29.6, t	29.7, t	31.4, t	31.5, t
8	126.3, s	126.2, s	42.6, d	42.6, d	47.7, d	47.5, d
9	135.9, s	136.2, s	53.1, d	53.0, d	55.3, d	55.3, d
10	37.8, s	37.9, s	36.8, s	36.8, s	36.9, s	36.9, s
11	27.6, t	28.9, t	30.6, t	30.8, t	27.5, t	28.7, t
12	70.2, d	71.1, d	71.4, d	71.4, d	50.3, d	54.5, d
13	135.0, s	135.5, s	135.4, s	135.3, s	83.5, d	82.0, d
14	149.0, d	148.7, d	148.7, d	148.8 d	153.3, s	148.5, s
15	175.9, s	174.9, s	174.9, s	174.9, s	108.1, t	110.3, t
16	73.9, t	72.5, t	72.4, t	72.3, t	65.7, t	20.4, q
17	69.2, t	69.9, t	101.9, d	101.8, d	28.6, q	28.6, q
18	28.4, q	28.4, q	28.5, q	28.5, q	17.0, q	17.0, q
19	20.6, q	19.5, q	18.0, q	18.0, q	14.0, q	13.9, q
20	17.1, q	17.1, q	14.7, q	14.7, q		
G1	105.6, d	105.6, d	105.6, d	105.5, d	105.6, d	105.6, d
G2	78.9, d	78.9, d	79.0, d	78.7, d	79.0, d	79.0, d
G3	79.4, d	79.4, d	79.5, d	79.3, d	79.5, d	79.5, d
G4	72.0, d	72.1, d	72.2, d	72.3, d	72.1, d	72.1, d
G5	77.6, d	77.6, d	77.6, d	74.9, d	77.6, d	77.6, d
G6	62.7, t	62.7, t	62.8, t	64.8, t	62.8, t	62.7, t
R1	101.8, d	101.8, d	101.8, d	101.9, d	101.9, d	101.8, d
R2	72.1, d	72.0, d	72.1, d	72.0, d	72.1, d	72.0, d
R3	72.1, d	72.0, d	72.1, d	72.0, d	72.0, d	72.0, d
R4	74.0, d	74.0, d	74.0, d	74.0, d	74.0, d	73.9, d
R5	70.0, d	70.0, d	70.0, d	70.0, d	70.0, d	70.0, d
R6	18.0, q	18.0, q	18.0, q	18.0, q	18.0, q	18.0, q
OAc				172.7, 20.8		

[a] G = glucose, R = rhamnose.

Compound **5** was obtained as a white powder, and the molecular formula was assigned to be $C_{31}H_{52}O_{12}$ by HRESIMS, which showed the molecular ion $[M]^+$ at *m*/*z* 616.3438. The IR spectrum of **5** had peaks at 3362 and 1038 cm^{-1}, suggestive of an oligoglycosidic moiety. In the ^{1}H and ^{13}C NMR and DEPT spectra, signals indicating three methyl groups, seven methylene groups (one olefinic methylene and one oxymethylene), six methines (two oxymethines δ_C 90.4, 83.5), four quaternary carbons (one olefinic carbon), and 12 carbon signals for two hexose moieties were found. Valence bond calculations revealed that the unsaturation degree of compound **5** was 6, indicating one double bond, two hexose moieties, and three rings.

The ^{1}H−^{1}H COSY spectrum of **5** showed cross-peaks of H-8/H-9/H-11/H-12/H-13, together with partial long-range correlations (C-3/H-G1, C-11/H-9, C-13/H-8) present in the HMBC spectrum, suggesting that **5** contained a 6−6−5 ring structure. On the basis of long-range correlations, OH groups were attached to C-13 (δ_C 83.5) and C-16 (δ_C 65.7), and an isopropenyl unit was located at C-12 (δ_C 50.3); a Rha-(1→2)-*O*-*β*-Glc was also attached at C-3. These data, together with the absence of a carbon atom compared with **3**, indicated **5** to be a norditerpene glycoside. On the basis of NOE correlations in the NOESY spectrum of **5**, the relative configuration of **5** was determined. The cross-peaks of H-3α/Me-17α, Me-18*β*/Me-19*β*, Me-19*β*/H-9*β*, H-9*β*/H-8*β*, H-8*β*/H-12*β*, and H-12*β*/H-13*β* and α-orientations were assigned to the oxygenated isopropenyl group at C-12 and to the OH group at C-13 in **5**. Thus, the structure of **5** was determined, and it was named noralpindenoside A.

Compounds **5** and **6** had similar IR and ^{1}H and ^{13}C NMR spectra. Detailed comparisons of the ^{1}H and ^{13}C NMR spectra of **5** with **6** indicated that the CH_2OH group (δ_C 65.7) at C-15 in **5** was replaced by a CH_3 group (δ_C 20.4) in **6**. Comparison

Table 3. Effects of **1**−**9**, **1a**, and **2a** on LPS-Induced NO Production in RAW264.7 Macrophages[a]

compound	IC_{50} (μM)
1	34.2
2	49.3
3	38.0
4	30.00
5	31.8
6	44.3
7	40.1
8	32.9
9	39.1
1a	12.2
2a	44.6
quercetin	9.6

[a] RAW264.7 cells were incubated with compounds in the presence of LPS (1 μg/mL). The medium was harvested 24 h later and assayed for nitrite production. NO released was measured using Griess reagent. Cell viability was evaluated with the MTT assay. The results are presented as a percentage of the control value obtained from nontreated cells. Values are expressed as mean ± SD of three individual experiments, performed in triplicate.

of the HRESIMS spectra of **5** and **6** indicated one less oxygen atom in **6**. HMBC experiments confirmed the OH group at C-13 and two sugars connected by Rha-(1→2)-*O*-β-Glc at C-3. These data, together with the NOE correlations, confirmed the structure of **6** (noralpindenoside B).

The other isolated compounds, **7**−**9**, were identified as kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranoside (**7**),[13] quercetin-3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranoside (**8**),[14] and morin-7-*O*-δ-D-glucopyranoside (**9**),[15] respectively, by comparison with literature data.

Compounds **1**−**9**, **1a**, and **2a** were evaluated for cytotoxicity against human tumor cell lines [HeLa (cervix epitheloid carcinoma), KB (oral epidermoid carcinoma), Doay (medulloblastoma), and WiDr (colon adenocarcinoma)] and anti-inflammatory activity, using RAW264.7 cells supplemented with LPS, which induced cell inflammation and caused nitrite accumulation in the medium. None of the isolates (**1**−**9**) nor **1a** and **2a** were cytotoxic against the above-mentioned tumor cells, and all of them had high cell viability (>80%) for RAW264.7 cells. Except for **1a**, all compounds showed only moderate anti-NO effects, with their IC_{50} values about 3 to 5 times that of quercetin (IC_{50} 9.6 μM). It was noted that **1a** (IC_{50} 12.2 μM) and **2a** (IC_{50} 44.6 μM) had significantly different inhibitory effects against NO production. These data implied that the configuration at C-12 of labdane diterpenoids plays a crucial role for the NO production induced by LPS.

As shown in Table 3, quercetin (positive control) had greater inhibition of LPS-induced NO production compared with flavonoid glycosides **7**−**9**. Moreover, the labdane diterpenoid **1a** had an IC_{50} value close to that of quercetin and exhibited much higher inhibitory activity than its glycoside, **1**. Sugar substituents may decrease the anti-inflammatory activity of flavonoid glycosides due to increased steric hindrance by or the hydrophilic character of additional sugar moieties, which may decrease the penetration and absorption of constituents into the cell. Several reports are consistent with the above conclusion.[16,17] In addition, comparing with quercetin (ED_{50} 18.0 μM), the isolated labdane diterpenoids (**1**−**6**) showed no antioxidative activity (ED_{50} >200 μg/mL) when assayed by DPPH. The active flavonoid glycosides **8** (ED_{50} 16.6 μM) and **9** (ED_{50} 22.2 μM) both have two OH groups in the B ring. Comparison of the inactive flavonoid glycoside **7**, together with a literature report,[18] indicates that additional OH groups in flavonoids increase their antioxidant activity.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO P-1020 polarimeter. IR spectra were measured on a Mattson Genesis II spectrophotometer (Thermo Nicolet, Madison, WI). NMR spectra were recorded on a Varian NMR spectrometer (Unity Plus 400 MHz) using CD_3OD or pyridine-d_5 as solvents. The chemical shifts are given in δ (ppm) and coupling constants in Hz. Low-resolution ESIMS were recorded on a VG Quattro 5022 mass spectrometer. High-resolution ESIMS were measured on a MAT-95XL high-resolution mass spectrometer. Sephadex LH-20 (Pharmacia) and silica gel (Merck 70−230 mesh and 230−400 mesh) were used for column chromatography, and precoated silica gel (Merck 60 F-254) plates were used for TLC. The spots on TLC were detected by spraying with 5% H_2SO_4 and then heating at 110 °C. HPLC separations were performed on a Waters 600 series apparatus with a Waters 996 photodiode array detector, equipped with a 250 × 10 mm i.d. preparative Cosmosil AR-II column (Nacalai, Tesque).

Plant Material. *Alpinia densespicata* was collected from YangMing Mountain of Taiwan in July 2003. A voucher specimen (No. 2003-07-066) has been deposited in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

Extraction and Isolation. Dried stems and leaves of *A. densespicata* (18 kg) were chipped, extracted with EtOH (80 L, three times) at 50 °C, and concentrated under reduced pressure. The EtOH extract (3.6 kg) was partitioned between *n*-hexane and H_2O (1:1) to give the *n*-hexane-soluble fraction (A, 350 g), $CHCl_3$ and H_2O (1:1) to give the $CHCl_3$-soluble fraction (B, 221 g), BuOH and H_2O (1:1) to give the BuOH-soluble fraction (C, 110 g), and the H_2O fraction, respectively. Fraction C (110 g) was chromatographed on a silica gel column (22 × 9 cm i.d.) eluted with $CHCl_3$−MeOH (1:0 → 1:1) to give 12 fractions (C-1 to C-12). Fraction C-11 (5 g, eluted with $CHCl_3$−MeOH, 5:1) was further separated on an LH-20 column (94 × 4 cm i.d.) eluted with $CHCl_3$−MeOH (1:1) to obtain fractions C-111 to C-117. Fraction C-113 (640 mg) was separated by HPLC (Cosmosil 5C_{18}-AR II, 250 × 10.0 mm i.d., flow rate: 2.5 mL/min, 60% MeOH) to yield **1** (345 mg) and **2** (92 mg). Fraction C-114 (133 mg) was separated by HPLC (Cosmosil C_{18}-AR II, 250 × 10.0 mm i.d., flow rate: 2.5 mL/min, 60% MeOH) to yield **3** (6.9 mg), **4** (6.0 mg), and **6** (13.5 mg). Fraction C-12 (6 g, $CHCl_3$−MeOH, 1:1) was repeatedly chromatographed on a LH-20 column (70 × 6 cm i.d.) with $CHCl_3$−MeOH (1:1) and then purified using preparative TLC ($CHCl_3$−MeOH (3:1)) to afford **5** (7.0 mg).

Alpindenoside A (1): white, amorphous powder; mp 168 °C; $[\alpha]^{24}_D$ −61.0 (*c* 1.00, MeOH); UV (MeOH) λ_{max}195.8 nm; IR (neat) ν_{max} 3387, 2937, 1746, 1656, 1445, 1356, 1209, 1042 cm^{-1}; ^1H NMR (MeOD, 600 MHz), Table 1; ^{13}C NMR (MeOD, 100 MHz), Table 2; HRESIMS *m/z* 663.3038 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_{13}Na$, 663.2993).

Alpindenoside B (2): white, amorphous powder; mp 162−165 °C; $[\alpha]^{24}_D$ −31.7 (*c* 0.60, MeOH); UV (MeOH) λ_{max} 195.8, 194.2 nm; IR (neat) ν_{max} 3373, 2933, 1739, 1630, 1380, 1205, 1042, 806 cm^{-1}; ^1H NMR (MeOD, 500 MHz), Table 1; ^{13}C NMR (MeOD, 100 MHz), Table 2; HRESIMS *m/z* 663.3048 $[M + Na]^+$ (calcd for $C_{32}H_{48}O_{13}Na$, 663.2993).

Alpindenoside C (3): white, amorphous powder; mp >295 °C; $[\alpha]^{24}_D$ −42.0 (*c* 0.69, MeOH); UV (MeOH) λ_{max} 195.6 nm; IR (neat) ν_{max} 3380, 2933, 1743, 1634, 1391, 1046, 813 cm^{-1}; ^1H NMR (MeOD, 500 MHz), Table 1; ^{13}C NMR (MeOD, 100 MHz), Table 2; HRESIMS *m/z* 681.3195 $[M + Na]^+$ (calcd for $C_{32}H_{50}O_{14}$ Na, 681.3200).

Alpindenoside D (4): white, amorphous powder; mp 240 °C; $[\alpha]^{24}_D$ −46.7 (*c* 0.60, MeOH); UV (MeOH) λ_{max} 195.6 nm; IR (neat) ν_{max} 3373, 2933, 1739, 1369, 1242, 1046, 810 cm^{-1}; ^1H NMR (MeOD, 500 MHz), Table 1; ^{13}C NMR (MeOD, 100 MHz), Table 2; HRESIMS *m/z* 723.3280 $[M + Na]^+$ (calcd for $C_{34}H_{52}O_{15}$ Na, 723.3204).

Noralpindenoside A (5): white, amorphous powder; mp 228 °C; $[\alpha]^{24}_D$ −28.6 (*c* 1.40, MeOH); UV (MeOH) λ_{max} 195.8 nm; IR (neat) ν_{max} 3362, 2930, 1568, 1402, 1038, 813 cm^{-1}; ^1H NMR (MeOD, 500 MHz), Table 1; ^{13}C NMR (MeOD, 100 MHz), Table 2; HREIMS *m/z* 616.3438 $[M]^+$ (calcd for $C_{31}H_{52}O_{12}$, 616.3459).

Noralpindenoside B (6): white, amorphous powder; mp >295 °C; $[\alpha]^{24}_D$ −34.8 (*c* 1.35, MeOH); UV (MeOH) λ_{max} 195.6 nm; IR (neat) ν_{max} 3380, 2933, 1391, 1042, 806 cm^{-1}; ^1H NMR (MeOD, 500 MHz), Table 1; ^{13}C NMR (MeOD, 100 MHz), Table 2; HRESIMS *m/z* 623.3476 $[M + Na]^+$ (calcd for $C_{31}H_{52}O_{11}Na$, 623.3407).

1a: $[\alpha]^{24}_D$ 264.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 210.0 nm; IR (neat) ν_{max} 3457, 2926, 2847, 2360, 1746, 1651, 1457, 1069 cm^{-1}; ^1H NMR ($CDCl_3$) 0.80 (3 H, s, H-18), 0.97 (3 H, s, H-19), 1.00 (3 H, s, H-20), 1.22 (1 H, m, H-5), 1.33 (1 H, m, H-1), 1.54 (1 H, ddd, J = 18.0, 12.6, 6.6, H-6), 1.63 (1 H, m, H-6), 1.71 (1 H, m, H-7), 1.76 (1 H, d, J = 7.2 Hz, H-1), 1.84 (1 H, m, H-2),1.96 (1 H, m, H-2), 1.90

(1 H, m, H-11), 2.47 (1 H, d, J = 14.4 Hz, H-11), 3.24 (1 H, dd, J = 4.2, 12.0 Hz, H-3), 3.95 (1 H, d, J = 15.0 Hz, H-17), 4.10 (1 H, d, J = 16.4 Hz, H-17), 4.34 (1 H, d, J = 7.8 Hz, H-12), 4.81 (1 H, s, H-16), 7.37 (1 H, s, H-14); ESIMS m/z 355.4 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4$).

2a: $[\alpha]^{24}_D$ 296.0 (c 0.5, MeOH); UV (MeOH) λ_{max} 210.0 nm; IR (neat) ν_{max} 3457, 2926, 2847, 2364, 1746, 1647, 1457, 1069 cm^{-1}; ^1H NMR ($CDCl_3$): 0.81 (3 H, s, H-18), 1.00 (3 H, s, H-19), 1.00 (3 H, s, H-20), 1.13 (1 H, d, J = 12.6 Hz, H-5), 1.18 (1 H, dd, J = 12.6, 3.0 Hz, H-1), 1.58 (1 H, m, H-2), 1.67(1 H, dd, J = 10.2, 4.8, H-1), 1.72 (1 H, m, H-6), 1.76 (1 H, dd, J = 6.0, 10.2, H-6), 1.85 (1 H, brd, H-7), 1.88 (1 H, brd, H-11), 2.38 (1 H, d, J = 16.8 Hz, H-11), 3.24 (1 H, dd, J = 3.0, 11.4 Hz, H-3), 3.99 (1 H, d, J = 15.0 Hz, H-17), 4.03 (1 H, d, J = 15.6 Hz, H-17), 4.23 (1 H, d, J = 10.2 Hz, H-12), 4.80 (1 H, s, H-16), 7.37 (1 H, s, H-14); ESIMS m/z 355.4 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_4$).

Acid Hydrolysis. Solutions of compounds **1** and **2** (each 20 mg) in 2 N HCl (2 mL) were refluxed at 100 °C for 3 h, respectively. Each reaction mixture was extracted with CH_2Cl_2 to yield the aglycone (**1a** or **2a**). After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and filtered. The filtrate was condensed, then dissolved in MeOH (0.2 mL) and purified by TLC in n-BuOH−MeOH−H_2O (8:8:1) to obtain the glucose and rhamnose. The sugars' specific optical rotations were compared with authentic samples. In addition, part of the filtrate was evaporated to dryness; then 1-(trimethylsilyl)imidazole and pyridine (0.2 mL) were added. After reacting for 1 h, the mixture was dried by a stream of N_2 and partitioned with n-hexane and water. The n-hexane layer was analyzed by GC with the following conditions: CP-Chirasil-L-Val column (25 m × 0.25 mm); injection temperature 200 °C; column temperature 100−200 °C; rate 3 °C/min. Peaks of trimethylsilyl derivatives derived from D-glucose (29.08 min) and L-rhamnose (16.74 min) were detected by comparison with retention times of authentic samples treated with 1-(trimethylsilyl)-imidazole.

NO Production and Cell Viability Assays. The murine macrophage cell line RAW264.7 (BCRC 60001 = ATCC TIB-71) was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and incubated at 37 °C in a humidified 5% CO_2 atmosphere using a 96-well flat-bottomed culture plate. After 24 h, the medium was replaced with fresh DMEM and FBS. Then compounds **1**−**9**, **1a**, and **2a** (0, 1, 5, 10, or 20 μg/mL) were added, respectively, in the presence of lipopolysaccharide (LPS, 1 μg/mL; Sigma, cat no. L-2654) and incubated at the same condition for 24 h. The cultured cells were then centrifuged, and the supernatants were used for NO production measurement. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was used to determine cell viability.

The supernatant was mixed with an equal volume of the Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% phosphoric acid solution) and incubated for 10 min at room temperature. Nitrite concentration was determined by measuring the absorbance at 540 nm using an ELISA plate reader (μ Quant).[19]

The MTT colorimetric assay was modified from that of Mosmann.[20] The test is based upon the selective ability of living cells to reduce the yellow soluble salt, MTT, to a purple-blue insoluble formazan. MTT (Merck; dissolved in phosphate-buffered saline at 5 mg/mL) solution was added to the attached cells mentioned above (10 μL per 100 μL culture) and incubated at 37 °C for 4 h. Then, DMSO was added and the amount of formazan formed was determined by absorbance at 550 nm. The optical density of formazan formed in the control (untreated) cells was taken as 100% viability.

Scavenging Activity of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical.[21] The radical scavenging activity of **1**−**9** on DPPH free radical was measured using the method of Rangkadilok et al.[22] and Chung et al.[23] with minor modifications. An aliquot of each sample (120 μL, 200−3.125 μg/mL) or quercetin (25−3.125 μg/mL) was mixed with 30 μL of 0.75 mM DPPH methanol solution in a 96-well microplate. The mixture was shaken vigorously on an orbital shaker in the dark at room temperature for 30 min, and then the absorbance at 517 nm was measured with an ELISA reader. The negative control was the measurement using methanol to replace the sample in the reaction solution. The DPPH radical scavenging activities of compounds **1**−**9** were compared to the negative control and to quercetin as a positive control. The final results were reported as ED_{50}, the concentration of sample required to cause 50% reduction of DPPH radicals in solution.

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Supporting Information Available: NMR spectra of **1**−**6**, including HMBC and NOESY spectra, and the presumed biosynthetic pathway of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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