Inhibitors of Nitric Oxide Production from the Rhizomes of *Alpinia galanga*: Structures of New 8–9′ Linked Neolignans and Sesquineolignan

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The 80% aqueous acetone extract from the rhizomes of *Alpinia galanga* showed nitric oxide (NO) production inhibitory activities in mouse peritoneal macrophages. From the aqueous acetone extract, three new 8–9′ linked neolignans, galanganal, galanganols A and B, and a sesquineolignan, galanganol C, were isolated together with nine known phenylpropanoids and *p*-hydroxybenzaldehyde. The structures of new neolignans were determined on the basis of physicochemical and chemical evidence. In addition, the inhibitory effects of the constituents from the rhizomes of *A. galanga* on NO production induced by lipopolysaccharide in mouse peritoneal macrophages were examined. Among them, galanganal (IC_{50}=68 μM), galanganols B (88 μM) and C (33 μM), 1′*S*-1′-acetoxychavicol acetate (2.3 μM), 1′*S*-1′-acetoxyeugenol acetate (11 μM), *trans*-*p*-hydroxycinnamaldehyde (*ca.* 20 μM), *trans*-*p*-coumaryl alcohol (72 μM), and *trans*-*p*-coumaryl diacetate (19 μM) were found to show inhibitory activity.

Key words *Alpinia galanga*; galanganal; galanganol; 8–9′ linked neolignan; sesquineolignan; nitric oxide production inhibitor

The Zingiberaceae plant *Alpinia galanga* SWARTZ is widely cultivated as a spice in South and Southeast Asian countries. The rhizomes of this plant are extensively used as a spice or ginger substitute for flavoring food, and also used as a stomachic in traditional Chinese medicine, or as a carminative, antiflatulent, antifungal, and anti-itching agent in traditional Thai medicine.[1,2] During the course of our characterization studies on Zingiberaceae natural medicines such as the rhizomes of *Zingiber officinale*,[3] *Curcuma zedoaria*,[4—7] and *Hedychium coronarium*,[8,9] and the fruit of *Alpinia oxyphylla*,[10] we have reported the gastroprotective[1] and antiallergic constituents[2] from the rhizomes of *A. galanga*. As a continuing study, we also found that the aqueous acetone extract from the rhizomes of *A. galanga* showed potent inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages. Through bioassay-guided separation, three new 8–9′ linked neolignans, galanganal (**1**) and galanganols A (**2**) and B (**3**), and a novel sesquineolignan, galanganol C (**4**), were isolated together with 10 known constituents.

In this paper, we describe the structure elucidation of four new neolignans (**1**—**4**) from the rhizomes of *A. galanga* as well as the inhibitory effects of the isolated constituents on NO production inhibitory activity.

The 80% aqueous acetone extract of the rhizomes of *A. galanga* was found to show NO production inhibitory activity in LPS-activated mouse peritoneal macrophages (IC_{50}=7.3 μg/ml). Through bioassay-guided separation, three new 8–9′ linked neolignans, **1** (0.0048% from the dried rhizomes), **2** (0.0011%), and **3** (0.0010%), and a sesquineolignan, **4** (0.0015%), were isolated together with 10 known constituents, 1′*S*-1′-acetoxychavicol acetate[11] (**5**, 1.10%), 1′*S*-1′-acetoxyeugenol acetate[11] (**6**, 0.038%), 1′*S*-1′-hydroxychavicol acetate[12] (**7**, 0.048%), chavicol β-D-glucopyranoside[13] (**8**, 0.023%), methyleugenol[14] (**9**, 0.0006%), *trans*-*p*-hydroxycinnamaldehyde[15] (**10**, 0.028%), *trans*-*p*-coumaryl alcohol[16] (**11**, 0.052%), *trans*-*p*-hydroxycinnamyl acetate[17] (**12**, 0.021%), *trans*-*p*-coumaryl diacetate[11] (**13**, 0.015%), and *p*-hydroxybenzaldehyde[14] (**14**, 0.0047%).

Structure of Galanganal (1) Galanganal (**1**) was isolated as a white powder. The molecular formula $C_{18}H_{16}O_3$ of **1** was determined from the molecular ion peak observed in the electron impact (EI)-MS and by high-resolution EI-MS measurement. The UV spectrum of **1** showed absorption maxima at 263 (log ε 4.14) and 314 (3.98), which were suggestive of a conjugated aromatic moiety.[18] The IR spectrum of **1** showed absorption bands at 3325, 1661, 1653, 1601, 1514, 1447, and 1173 cm^{-1} ascribable to hydroxyl, conjugated aldehyde, and conjugated olefin functions and aromatic ring. The ^{1}H- and ^{13}C-NMR (CD_3OD, Table 1) spectra of **1** showed signals due to a methylene [δ 3.31 (1H, m), 3.39 (1H, dd-like), 9′-H_2], a *trans*-olefin pair, and a trisubstituted olefin [δ 6.10 (1H, ddd, J=5.8, 5.8, 15.8 Hz, 8′-H), 6.28 (1H, d, J=15.8 Hz, 7′-H), 7.44 (1H, s, 7-H)], two *para*-substituted aromatic rings [δ 6.68, 7.14 (2H each, both d, J=8.6 Hz, 3′,5′-H, 2′,6′-H), 6.86, 7.53 (2H each, both d, J=8.8 Hz, 3,5-H, 2,6-H)], and an aldehyde group [δ 9.52 (1H, s, 9-H)]. The 8–9′ linked neolignan skeleton in **1** was constructed on the basis of homo- and heterocorrelation spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple-bond connectivity (HMBC) experiments. The ^{1}H–^{1}H COSY experiment on **1** indicated the presence of partial structures, shown by bold lines in Fig. 1. In the HMBC experiment of **1**, long-range correlations were observed between the following proton and carbon pairs: 2,6-H and 4,7-C; 3,5-H and 1,4-C; 7-H and 1,2,6,9,9′-C; 9-H and 7,8,9′-C; 2′,6′-H and 4′,7′-C; 3′,5′-H and 1′,4′-C; 7′-H and 1′,2′,6′-C; 8′-H and 1′-C; 9′-H_2 and 7,8,9-C (Fig. 1). The geometry of the 7–8 position in **1** was determined in a nuclear Overhauser enhancement spectroscopy (NOESY) experiment, in which a NOE correlation was observed between the 7-proton and 9-proton, as shown in Fig. 1. The evidence led us to designate the structure of galanganal as shown to be **1**.

Structures of Galanganols A (2) and B (3) Galanganols A (**2**) and B (**3**) were obtained as a white pow-

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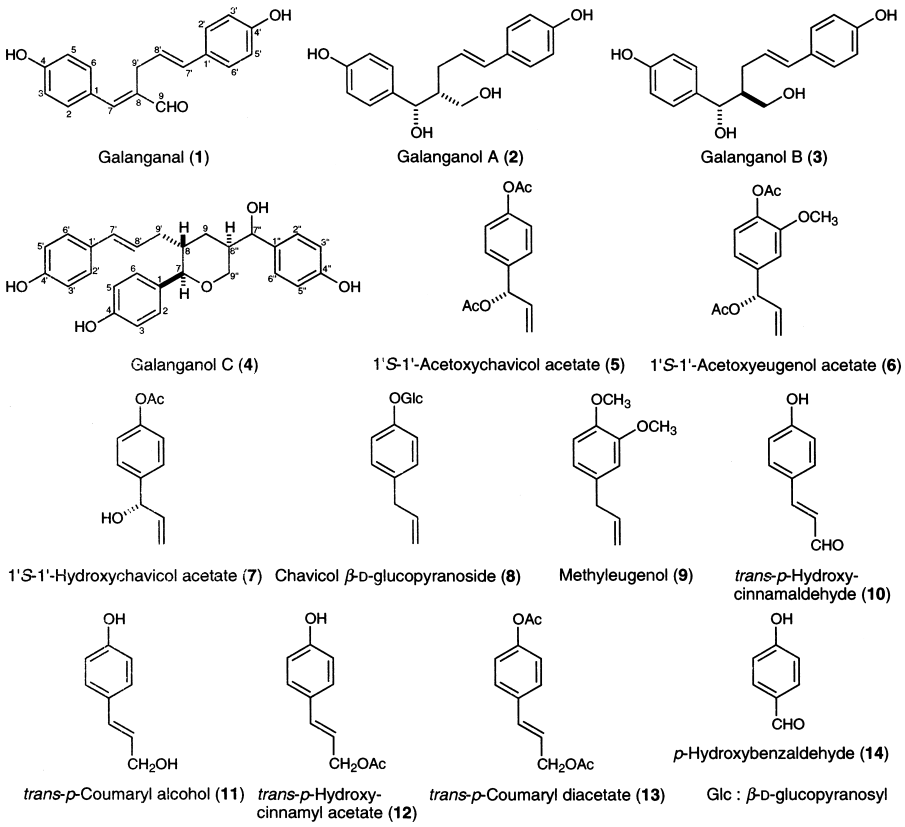

Chart 1

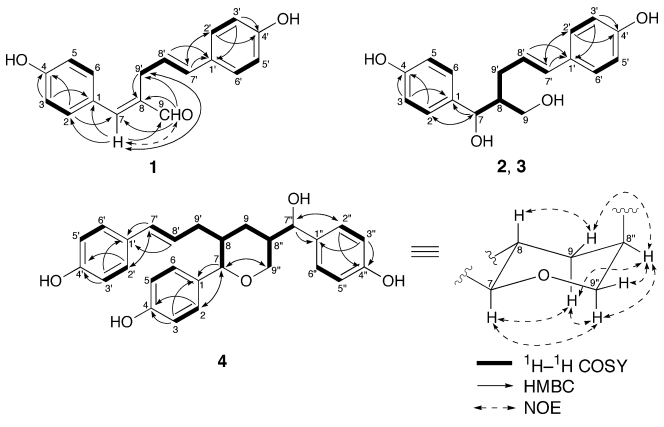

Fig. 1. ^{1}H–^{1}H COSY, HMBC, and NOE Correlations of **1**—**4**

der and their molecular formulas were determined by EI-MS and high-resolution EI-MS measurements to be $C_{18}H_{20}O_4$, respectively. The UV spectra of **2** and **3** showed an absorption maxmum [**2**, 262 nm (log ε 4.33); **3**, 262 (4.35)], while their IR spectra showed absorption bands due to hydroxyl and olefin functions and aromatic ring (**2**, 3649, 1653, 1559, 1509, 1458 cm^{-1}; **3**, 3649, 1653, 1559, 1507, 1458 cm^{-1}). The proton and carbon signals in the ^{1}H- and ^{13}C-NMR (CD_3OD, Table 1) of **2** showed signals assignable to a methylene and a methine [δ 1.92 (1H, m, 8-H), 2.23, 2.33 (1H each, both m, 9′-H_2)], a methylene and a methine bearing an oxygen function {δ [3.40 (1H, dd, J=5.3, 10.9 Hz), 3.56 (1H, dd, J=6.0, 10.9 Hz), 9-H_2], 4.74 (1H, d, J=5.9 Hz, 7-H)}, a *trans*-olefin pair [δ 6.00 (1H, ddd, J=6.9, 7.7, 16.0 Hz, 8′-H), 6.28 (1H, d, J=16.0 Hz, 7′-H)], and two

Table 1. ^{13}C-NMR Data of Galanganal (**1**) and Galanganols A—C (**2**—**4**)

	1[a]	**2**[a]	**3**[a]	**4**[b]
C-1	127.5	135.6	135.5	133.3
C-2,6	133.7	128.4	128.9	129.5
C-3,5	116.8	115.7	115.8	116.0
C-4	161.1	157.3	157.6	158.6
C-7	153.7	74.5	76.3	85.7
C-8	138.4	49.5	48.8	38.8
C-9	197.2	62.6	63.1	31.8
C-1′	130.5	130.8	130.6	129.8
C-2′,6′	128.3	127.9	127.9	127.9
C-3′,5′	116.3	116.0	116.0	116.4
C-4′	157.9	157.3	157.3	158.2
C-7′	131.8	132.2	132.3	131.5
C-8′	123.9	126.6	126.1	125.4
C-9′	28.6	30.5	32.3	35.9
C-1″				136.6
C-2″,6″				128.7
C-3″,5″				123.8
C-4″				158.2
C-7″				72.4
C-8″				43.4
C-9″				69.1

Measured in *a*) CD_3OD and *b*) pyridine-d_5 at 125 MHz.

para-substituted aromatic rings [δ 6.68, 7.14 (2H each, both d, J=8.6 Hz, 3′,5′-H, 2′,6′-H), 6.76, 7.18 (2H each, both d, J=8.6 Hz, 3,5-H, 2,6-H)]. In addition, the proton and carbon signals in the ^{1}H- and ^{13}C-NMR (Table 1) spectra of **3** were similar to those of **2** {a methylene and a methine [δ 1.92 (1H, m, 8-H), 2.01, 2.10 (1H each, both m, 9′-H_2)], a methylene and a methine bearing an oxygen function {δ [3.65 (1H, dd,

J=5.3, 11.1 Hz), 3.78 (1H, dd, J=4.8, 11.1 Hz), 9-H_2], 4.62 (1H, d, J=7.1 Hz, 7-H)}, a *trans*-olefin pair [δ 5.90 (1H, ddd, J=6.8, 7.2, 15.8 Hz, 8′-H), 6.19 (1H, d, J=15.8 Hz, 7′-H)], and two *para*-substituted aromatic rings [δ 6.68, 7.12 (2H each, both d, J=8.4 Hz, 3′,5′-H, 2′,6′-H), 6.77, 7.18 (2H each, both d, J=8.4 Hz, 3,5-H, 2,6-H)]}. As shown in Fig. 1, the ^{1}H–^{1}H COSY experiment on **2** and **3** indicated the presence of partial structures drawn in bold lines. In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs of **2** and **3** (2,6-H, 4,7-C; 3,5-H, 1,4-C; 7-H, 1,2,6-C; 2′,6′-H, 4′,7′-C; 3′,5′-H, 1′,4′-C; 7′-H, 1′,2′,6′-C; 8′-H, 1′-C), and thus the planar structure of **2** and **3** were determined to be as shown.

Next, the stereostructures of **2** and **3** were clarified by comparison of the ^{1}H–^{1}H coupling constant between the 7-proton and the 8-proton pairs in the 7,8-acetonide derivatives (**2a**, **3a**). Treatment of **2** or **3** with 2,2-dimethoxypropane gave the 7,8-acetonide derivatives (**2a** or **3a**), respectively. As shown in Fig. 2, the coupling constants in the ^{1}H-NMR spectrum of **2a** showed $J_{7,8}$=2.8 Hz, $J_{8,9ax}$=2.4 Hz, and $J_{8,9eq}$= 1.5 Hz, and thus the stereostructure of **2** was determined to be a 7,8-*erythro*-form. On the other hand, the stereostructure of the 7–8 positions in **3** was elucidated to be a 7,8-*threo*-form ($J_{7,8}$=10.1 Hz, $J_{8,9ax}$=9.8 Hz, and $J_{8,9eq}$=<1 Hz).[19,20]

Structure of Galanganol C (4) Galanganol C (**4**) was obtained as a white powder and its UV spectrum showed an absorption maximum at 263 nm (log ε 4.31). The IR spectrum of **4** showed absorption bands at 3650, 3260, 1654, 1609, 1516, 1456, 1229, 1171, and 1043 cm^{-1} ascribable to hydroxyl, olefin, and ether functions and aromatic ring. The EI-MS spectrum of **4** showed a molecular ion peak at *m*/*z* 432 (M^+) together with fragment ion peaks at *m*/*z* 414, 107, and 94 and the molecular formula $C_{27}H_{28}O_5$ of **4** was elucidated by high resolution EI-MS measurement. The ^{1}H- and ^{13}C-NMR (pyridine-d_5, Table 1) spectra of **4** showed signals assignable to two methylenes and two methines {δ 1.45 (1H, ddd, J=4.9, 13.4, 13.7 Hz, 9_{ax}-H), [1.82 (1H, ddd, J=7.5, 7.6, 14.0 Hz), 2.14 (1H, m), 9′-H_2], 1.89 (1H, br dd, J=*ca.* 3, 14 Hz, 9_{eq}-H), 2.14, 2.30 (1H each, both m, 8″-H, 8-H)}, a methylene and two methines bearing an oxygen function [δ 3.86 (1H, dd, J=2.5, 11.3 Hz, $9''_{ax}$-H), 4.19 (1H, d, J=9.7 Hz, 7-H), 5.04 (1H, br d, J=*ca.* 11 Hz, $9''_{eq}$-H), 5.51 (1H, d, J=10.4 Hz, 7″-H)], a *trans*-olefin pair [δ 5.89 (1H, ddd, J=7.3, 7.6, 15.9 Hz, 8′-H), 6.26 (1H, d, J=15.9 Hz, 7′-H)], and three *para*-substituted aromatic rings [δ 7.15, 7.32 (2H each, both d, J=8.6 Hz, 3′,5′-H, 2′,6′-H), 7.20, 7.61 (2H each, both d, J=8.6 Hz, 3″,5″-H, 2″,6″-H), 7.24, 7.56 (2H each, both d, J=8.6 Hz, 3,5-H, 2,6-H)]. The ^{1}H–^{1}H COSY and the HBMC experiments on **4** indicated the presence of ^{1}H–^{1}H and ^{1}H–^{13}C long range correlations, as shown in Fig. 1. The stereostructure of the the tetrahydropyran moiety in **4** was determined in a NOESY experiment, in which NOE correlations were observed between 7-H and 9_{ax}-H, $9''_{ax}$-H; 8-H and 9_{eq}-H; 8″-H and 9-H_2, 9″-H_2, as shown in Fig. 1. This evidence was also suggested by the coupling constants in the ^{1}H-NMR spectrum on **4** ($J_{7,8}$=9.7 Hz and $J_{8'',9''_{ax}}$=2.5 Hz). On the basis of these findings, the structure of **4** was determined to be as shown.[19]

The class of neolignans is widely distributed in various plants. However, the isolation reports of 8–9′ linked neolignans (*e.g.*, **1**—**3**) are rare, and also, 8–9′–8″ linked sesquineolignans with a tetrahydropyrane ring (*e.g.*, **4**) have been isolated from only one plant material, *Morina chinensis*.[21,22]

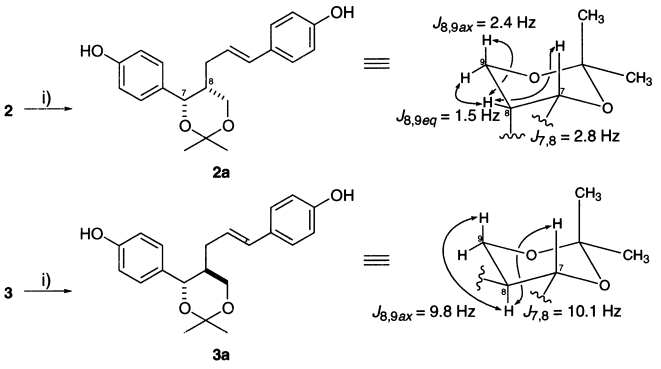

Fig. 2

Inhibitory Effects on NO Production in LPS-Activated Mouse Peritoneal Macrophages The inorganic free radical NO has been implicated in physiologic and pathologic processes, such as vasodilation, nonspecific host defense, ischemia-reperfusion injury, and chronic or acute inflammation. NO is produced by the oxidation of L-arginine by NO synthase (NOS). In the family of NOS, inducible NOS (iNOS) is specifically involved in pathologic aspects with the overproduction of NO and can be expressed in response to proinflammatory agents such as interleukin-1β, tumor necrosis factor-α, and LPS in various cell types including macrophages, endothelial cells, and smooth muscle cells. As a part of our studies to characterize the bioactive components of natural medicines, we have investigated various NO production inhibitors, *i.e.*, higher unsaturated fatty acids,[23] polyacetylenes,[24,25] coumarins,[24,26] flavonoids,[25,27] stilbenes,[28,29] lignans,[30,31] sesquiterpenes,[6,10,32—36] diterpenes,[8,37] triterpenes,[38—41] diarylheptanoids,[42,43] cyclic peptides,[40] and alkaloids.[44,45]

In the previous study, 1′*S*-1′-acetoxychavicol acetate (**5**) from *A. galanga* (syn. *Languas galanga*) was reported to inhibit NO production strongly in a macrophage-like cell line, RAW265,[46] while the effects of the other constituents were not reported. Therefore the effects of the constituents (**1**—**14**) from the rhizomes of *A. galanga* on NO production in LPS-activated macrophages were examined. As shown in Table 2, 1′*S*-1′-acetoxychavicol acetate (**5**, IC_{50}=2.3 μM) exhibited the strongest activity among the isolated constituents, in agreement with the previous report.[46] However, not only **5** but also galanganal (**1**, IC_{50}=68 μM), galanganols B (**3**, 88 μM) and C (**4**, 33 μM), 1′*S*-1′-acetoxychavicol acetate (**5**, 2.3 μM), 1′*S*-1′-acetoxyeugenol acetate (**6**, 11 μM), *trans*-*p*-hydroxycinnamaldehyde (**10**, *ca.* 20 μM), *trans*-*p*-coumaryl alcohol (**11**, 72 μM), and *trans*-*p*-coumaryl diacetate (**13**, 19 μM) showed substantial inhibition without cytotoxic effects in the MTT assay, and the inhibitory activities of **4**—**6**, **10**, and **13** were stronger than that of an NO synthase inhibitor [N^G-monomethyl-L-arginine (L-NMMA)] or an inhibitor of nuclear factor-κB activation [caffeic acid phenetyl ester (CAPE)].[47] In addition, the structure–activity relationships of **5** in the inhibitory effects on NO production was described in our report.[48]

Table 2. Inhibitory Effects of Constituents from *A. galanga* on LPS-Activated NO Production in Mouse Peritoneal Macrophages

	Inhibition (%)						IC_{50} (μM)
	0 μM	1 μM	3 μM	10 μM	30 μM	100 μM	
Galanganal (**1**)	0.0±1.2	12.7±5.8*	−7.1±2.9	−1.2±2.1	11.0±2.4	68.2±2.3**	68
Galanganol A (**2**)	0.0±3.7	−1.2±5.0	1.3±6.6	−4.6±3.5	17.7±4.9*	38.9±2.1**	
Galanganol B (**3**)	0.0±2.9	1.2±4.4	6.1±2.4	12.2±3.1*	24.3±4.9**	54.3±2.8**	88
Galanganol C (**4**)	0.0±2.0	−4.4±4.1	−1.1±4.0	5.8±4.3	41.9±6.0**	96.6±0.9**	33
1′*S*-1′-Acetoxychavicol acetate (**5**)	0.0±1.1	17.6±1.1**	59.9±1.6**	97.5±0.5**[a)]			2.3
1′*S*-1′-Acetoxyeugenol acetate (**6**)	0.0±6.0	−10.8±2.8	5.8±5.6	40.4±1.7**	90.7±0.8**		11
1′*S*-1′-Hydroxychavicol acetate (**7**)	0.0±2.3	6.1±2.9	3.8±3.3	5.2±3.1	14.5±3.2*	45.6±2.9**	
Chavicol β-D-glucopyranoside (**8**)	0.0±3.0	−6.1±2.9	1.4±2.7	−5.7±6.5	1.1±3.3	5.9±2.0	
Methyleugenol (**9**)	0.0±8.5	−1.6±3.7	−4.0±3.6	−5.0±1.8	5.2±1.2	1.0±3.4	
trans-p-Hydroxycinnamaldehyde (**10**)	0.0±1.6	13.4±7.4	−8.2±8.4	−0.5±10.7	78.4±1.1**	100.3±1.1**	*ca.* 20
trans-p-Hydroxycinnamyl acetate (**11**)	0.0±4.0	4.0±3.0	7.5±1.6	17.4±5.0**	24.9±1.2**	61.0±2.9**	72
trans-p-Coumaryl alcohol (**12**)	0.0±0.2	2.4±1.5	4.7±0.7	5.2±4.4	9.9±1.3*	23.6±3.0**	
trans-p-Coumaryl diacetate (**13**)	0.0±7.5	−0.3±6.0	−0.2±12.3	26.7±14.3	76.8±12.6**	96.0±1.7**	19
p-Hydroxybenzaldehyde (**14**)	0.0±2.8	5.2±3.6	0.9±2.2	1.9±0.5	8.7±7.6	17.2±2.9*	
L-NMMA	0.0±4.0	5.9±0.9	10.3±3.7	15.0±1.6**	34.1±3.2**	63.1±1.2**	57
CAPE	0.0±0.7	3.8±0.1	1.4±0.1	68.2±0.0**	93.7±0.2**	99.6±0.0**[a)]	15

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, * $p<0.05$, ** $p<0.01$. *a*) Cytotoxic effect was observed.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index detector.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150—350 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The rhizome of *A. galanga* was collected in Thailand in July 2000, and was identified by Dr. Yutana Pongpiriyadacha (Faculty of Agriculture Nakhon si Thammarat, Rajamangala Institute of Technology). A voucher specimen (No. T-04) for this natural medicine is on file in our laboratory.

Extraction and Isolation The dried rhizomes of *A. galanga* (2.1 kg) were powdered and extracted three times with 80% aqueous acetone at room temperature for 1 d. Evaporation of the solvent under reduced pressure provided an aqueous acetone extract (138 g, 6.6% from the dried rhizomes). Normal-phase silica gel column chromatography [3.0 kg, *n*-hexane–EtOAc (10 : 1→5 : 1, v/v)→EtOAc→MeOH] of the acetone extract (130 g) gave 13 fractions {fr. 1 (0.7 g), 2 (2.3 g), 3 (0.5 g), 4 (2.8 g), 5 [=1′*S*-1′-acetoxychavicol acetate (**5**, 23.0 g, 1.10%)], 6 (1.7 g), 7 (2.6 g), 8 (3.6 g), 9 (1.9 g), 10 (5.8 g), 11 (5.0 g), 12 (6.3 g), 13 (73.8 g)}. Fraction 3 (0.5 g) was separated by reverse-phase silica gel column chromatography [15 g, MeOH–H_2O (70 : 30→90 : 10, v/v)→MeOH] to give methyleugenol (**9**, 14 mg, 0.0006%). Fraction 6 (350 mg) was separated by preparative HPLC [CH_3CN–H_2O (40 : 60, v/v)] to give *trans-p*-coumaryl diacetate (**13**, 63 mg, 0.015%) and 1′*S*-1′-acetoxyeugenol acetate (**6**, 131 mg, 0.032%). Fraction 7 (2.6 g) was separated by reverse-phase silica gel column chromatography [80 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give *p*-hydroxybenzaldehyde (**14**, 99 mg, 0.0047%), 1′*S*-1′-hydroxychavicol acetate (**7**, 1005 mg, 0.048%), *trans-p*-hydroxycinnamyl acetate (**11**, 443 mg, 0.021%), and **6** (139 mg, 0.0066%). Fraction 8 (3.6 g) was separated by reverse-phase silica gel column chromatography [100 g, MeOH–H_2O (25 : 75→50 : 50, v/v)→MeOH] to give *trans-p*-hydroxycinnamaldehyde (**10**, 578 mg, 0.028%). Fraction 9 (1.9 g) was separated by reversed-phase silica gel column chromatography [60 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] to give *trans-p*-coumaryl alcohol (**11**, 1090 mg, 0.052%) and galanganal (**1**, 100 mg, 0.0048%). Fraction 11 (5.0 g) was subjected to reverse-phase silica gel column chromatography [150 g, MeOH–H_2O (40 : 60→50 : 50→60 : 40→70 : 30→80 : 20, v/v)→MeOH] to furnish seven fractions [fr. 11-1 (828 mg), 11-2 (696 mg), 11-3 (379 mg), 11-4 (1169 mg), 11-5 (911 mg), 11-6 (351 mg), and 11-7 (666 mg)]. Fraction 11-2 (696 mg) was subjected to HPLC [MeOH–H_2O (50 : 50, v/v)] to give galanganols A (**2**, 24 mg, 0.0011%) and B (**3**, 21 mg, 0.0010%). Fraction 11-4 (1169 mg) was further purified by HPLC [MeOH–H_2O (60 : 40, v/v)] to give galanganol C (**4**, 32 mg, 0.0015%). Fraction 12 (6.3 g) was separated by reverse-phase silica gel column chromatography [200 g, MeOH–H_2O (25 : 75→40 : 60→60 : 40, v/v)→MeOH] to give chavicol β-D-glucopyranoside (**8**, 476 mg, 0.023%).

Galanganal (**1**): A white powder. High-resolution EI-MS: Calcd for $C_{18}H_{16}O_3$ (M^+) 280.1099; Found 280.1094. UV [MeOH, nm (log ε)]: 263 (4.14), 314 (3.98). IR (KBr): 3325, 1661, 1653, 1601, 1514, 1447, 1173 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: [3.31 (1H, m), 3.39 (1H, dd-like), 9′-H_2], 6.10 (1H, ddd, J=5.8, 5.8, 15.8 Hz, 8′-H), 6.28 (1H, d, J=15.8 Hz, 7′-H), 6.68, 7.14 (2H each, both d, J=8.6 Hz, 3′,5′-H, 2′,6′-H), 6.86, 7.53 (2H each, both d, J=8.8 Hz, 3,5-H, 2,6-H), 7.44 (1H, s, 7-H), 9.52 (1H, s, 9-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: see Table 1. EI-MS: m/z 280 (M^+, 100), 251 (M^+-CHO, 40), 186 ($M^+-C_6H_6O$, 17), 173 ($M^+-C_7H_7O$, 97), 120 ($C_8H_8O^+$, 28), 107 ($C_7H_7O^+$, 42).

Galanganol A (**2**): A white powder, $[\alpha]_D^{20}$ ±0° (c=0.34, MeOH). High-resolution EI-MS: Calcd for $C_{18}H_{20}O_4$ (M^+) 300.1361; Found 300.1366. UV [MeOH, nm (log ε)]: 262 (4.33). IR (KBr): 3649, 1653, 1559, 1509, 1458 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.92 (1H, m, 8-H), 2.23, 2.33 (1H each, both m, 9′-H_2), [3.40 (1H, dd, J=5.3, 10.9 Hz), 3.56 (1H, dd, J=6.0, 10.9 Hz), 9-H_2], 4.74 (1H, d, J=5.9 Hz, 7-H), 6.00 (1H, ddd, J=6.9, 7.7, 16.0 Hz, 8′-H), 6.28 (1H, d, J=16.0 Hz, 7′-H), 6.68, 7.14 (2H each, both d, J=8.6 Hz, 3′,5′-H, 2′,6′-H), 6.76, 7.18 (2H each, both d, J=8.6 Hz, 3,5-H, 2,6-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: see Table 1. EI-MS: m/z 300 (M^+, 4), 282 (M^+-H_2O, 14), 252 (44), 158 (27), 145 (62), 131 (50), 120 ($C_8H_8O^+$, 49), 107 ($C_7H_7O^+$, 100).

Galanganol B (**3**): A white powder, $[\alpha]_D^{22}$ ±0° (c=0.96, MeOH). High-resolution EI-MS: Calcd for $C_{18}H_{20}O_4$ (M^+) 300.1361; Found 300.1358. UV [MeOH, nm (log ε)]: 262 (4.35). IR (KBr): 3649, 1653, 1559, 1507, 1458 cm^{-1}. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.92 (1H, m, 8-H), 2.01, 2.10 (1H each, both m, 9′-H_2), [3.65 (1H, dd, J=5.3, 11.1 Hz), 3.78 (1H, dd, J=4.8, 11.1 Hz), 9-H_2], 4.62 (1H, d, J=7.1 Hz, 7-H), 5.90 (1H, ddd, J=6.8, 7.2, 15.8 Hz, 8′-H), 6.19 (1H, d, J=15.8 Hz, 7′-H), 6.68, 7.12 (2H each, both d, J=8.4 Hz, 3′,5′-H, 2′,6′-H), 6.77, 7.18 (2H each, both d, J=8.4 Hz, 3,5-H, 2,6-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: see Table 1. EI-MS: m/z 300 (M^+, 1), 282 (M^+-H_2O, 6), 252 (84), 158 (46), 145 (95), 131 (49), 120 ($C_8H_8O^+$, 24), 107 ($C_7H_7O^+$, 100).

Galanganol C (**4**): A white powder, $[\alpha]_D^{28}$ ±0° (c=0.18, MeOH). High-resolution EI-MS: Calcd for $C_{27}H_{28}O_5$ (M^+) 432.1937; Found 432.1934. UV [MeOH, nm (log ε)]: 263 (4.31). IR (KBr): 3650, 3260, 1654, 1609, 1516, 1456, 1229, 1171, 1043 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.45 (1H, ddd, J=4.9, 13.4, 13.7 Hz, 9_{ax}-H), [1.82 (1H, ddd, J=7.5, 7.6, 14.0 Hz), 2.14

(1H, m), 9′-H_2], 1.89 (1H, br dd, J=*ca.* 3, 14 Hz, 9_{eq}-H), 2.14 (1H, m, 8″-H), 2.30 (1H, m, 8-H), 3.86 (1H, dd, J=2.5, 11.3 Hz, $9''_{ax}$-H), 4.19 (1H, d, J=9.7 Hz, 7-H), 5.04 (1H, br d, J=*ca.* 11 Hz, $9''_{eq}$-H), 5.51 (1H, d, J=10.4 Hz, 7″-H), 5.89 (1H, ddd, J=7.3, 7.6, 15.9 Hz, 8′-H), 6.26 (1H, d, J=15.9 Hz, 7′-H), 7.15, 7.32 (2H each, both d, J=8.6 Hz, 3′,5′-H, 2′,6′-H), 7.20, 7.61 (2H each, both d, J=8.6 Hz, 3″,5″-H, 2″,6″-H), 7.24, 7.56 (2H each, both d, J=8.6 Hz, 3,5-H, 2,6-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: see Table 1. EI-MS: m/z 432 (M^+, 1), 414 (M^+-H_2O, 2), 107 ($C_7H_7O^+$, 23), 94 ($C_6H_6O^+$, 100).

Preparation of the Acetonide Derivatives (2a, 3a) from Galanganols A (2) and B (3) A solution of **2** or **3** (each 0.9 mg, 3.0 μmol) in acetone (0.5 ml) was treated with 2,2-dimethoxypropane (0.1 ml) and Dowex HCR-W2 (H^+ form, 5—10 grains), and the mixture was stirred at room temperature for 12 h, respectively. The resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure gave a residue that was purified by silica gel column chromatography (0.1 g, *n*-hexane–AcOEt=1 : 5, v/v) to furnish **2a** (1.0 mg, 98%) or **3a** (0.7 mg, 69%), respectively.

2a: A white powder. High-resolution EI-MS: Calcd for $C_{21}H_{24}O_4$ (M^+) 340.1674; Found 340.1676. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.48, 1.55 (3H each, both s, $-CH_3$), 1.67 (1H, m, 8-H), 1.83, 2.37 (1H each, both m, 9′-H_2), 3.86 (1H, dd, J=1.5, 11.6 Hz, 9_{eq}-H), 4.22 (1H, dd, J=2.4, 11.6 Hz, 9_{ax}-H), 5.24 (1H, d, J=2.8 Hz, 7-H), 5.78 (1H, ddd, J=6.4, 8.9, 15.9 Hz, 8′-H), 6.18 (1H, d, J=15.9 Hz, 7′-H), 6.65, 7.09 (2H each, both d, J=8.5 Hz, 3′,5′-H, 2′,6′-H), 6.76, 7.14 (2H each, both d, J=8.9 Hz, 3,5-H, 2,6-H). EI-MS: m/z 340 (M^+, 22), 282 (85), 252 (21), 160 (100).

3a: A white powder. High-resolution EI-MS: Calcd for $C_{21}H_{24}O_4$ (M^+) 340.1674; Found 340.1670. ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.37, 1.54 (3H each, both s, $-CH_3$), 1.85, 1.94 (1H each, both m, 9′-H_2), 1.96 (1H, m, 8-H), 3.81 (1H, br d, J=*ca.* 12 Hz, 9_{eq}-H), 3.88 (1H, dd, J=9.8, 11.9 Hz, 9_{ax}-H), 4.55 (1H, d, J=10.1 Hz, 7-H), 5.75 (1H, ddd, J=6.4, 8.6, 15.9 Hz, 8′-H), 6.15 (1H, d, J=15.9 Hz, 7′-H), 6.78, 7.08 (2H each, both d, J=8.8 Hz, 3′,5′-H, 2′,6′-H), 6.90, 7.22 (2H each, both d, J=8.5 Hz, 3,5-H, 2,6-H). EI-MS: m/z 340 (M^+, 5), 282 (73), 252 (37), 160 (100).

Bioassay. NO Production from LPS-Stimulated Macrophages Inhibitory effects on the NO production by mouse macrophages were evaluated using the method reported previously.[41,45] Briefly, peritoneal exudate cells were collected from the peritoneal cavities of male ddY mice, which had been intraperitoneally injected with 4% thioglycolate medium 4 d before, and washed with 6—7 ml of ice-cold phosphate-buffered saline (PBS). The cells (5×10^5 cells/well) were suspended in 100 μl of RPMI 1640 supplemented with 5% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml), and precultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing the cells with PBS, and the adherent cells were cultured in fresh medium containing LPS 10 μg/ml and test compound (1—100 μM) for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite in the culture medium using Griess reagent. Cytotoxicity was determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay. Briefly, after 20-h incubation with test compounds, MTT (10 μl, 5 mg/ml in PBS) solution was added to the wells. After 4-h culture, the medium was removed, and isopropanol containing HCl 0.04 M was then added to dissolve the formazan produced in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm (reference, 655 nm). When the optical density of the sample-treated group was less than 80% of that in the vehicle-treated group, the test compound was considered to exhibit cytotoxic effects. L-NMMA and CAPE were used as reference compounds. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final DMSO concentration 0.5%). Inhibition (%) was calculated using the following formula and the IC_{50} was determined graphically (n=4):

$$\text{inhibition (\%)} = \frac{A-B}{A-C}\times 100$$

A—C: NO_2^- concentration (μM) [A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)].

Statistics Values are expressed as mean±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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20) The plane structure of galanganols A (**2**) and B (**3**) is identical with that proposed for (4*E*)-1,5-bis(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol, which was isolated from *Alpinia officinarum*.[18]
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