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

Toshio MORIKAWA,*‡ Shin ANDO,‡ Hisashi MATSUDA,* Shinya KATAOKA,* Osamu MURAOKA,‡ and Masayuki YOSHIIWA*,*

*Kyoto Pharmaceutical University; Mmisaiga, Yamashina-ku, Kyoto 607–8412, Japan: and ‡School of Pharmaceutical Sciences, Kinki University; 3–4–1 Kwakake, Higashiosaka, Osaka 577–8502, Japan.

Received January 5, 2005; accepted February 21, 2005

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 (IC50 = 68 μM), galanganols B (88 μM) and C (33 μM), 1'S-1'-aceoxychavicol acetate (2.3 μM), 1'S-1'-aceoxygeugenol 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, anti-inflammatory, 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 *Hedychoicium coronarium*,8,9) and the fruit of *Alpinia oxyphylla*,10) we have reported the gastroprotective11) and antiallergic constituents2) 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 (IC50 = 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’-aceoxychavicol acetate (5) (1.10%), 1’S-1’-aceoxygeugenol acetate (6) (0.038%), 1’S-1’-hydroxychavicol acetate (7) (0.048%), chavicol β-o-glucopyranoside (8) (0.023%), myrteyleugenol (9) (0.0006%), trans-p-hydroxycinnamaldehyde (10) (0.028%), trans-p-coumaryl alcohol (11) (0.052%), trans-p-hydroxycinnamyl acetate (12) (0.021%), trans-p-coumaryl diacetate (13) (0.015%), and p-hydroxybenzaldehyde (14) (0.0047%).

**Structure of Galanganal (1)** Galanganal (1) was isolated as a white powder. The molecular formula C18H16O3 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.10) 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 1H- and 13C-NMR (CD3OD, Table 1) spectra of 1 showed signals due to a methylene [δ 3.31 (1H, m), 3.39 (1H, dd-like), 9’-H], 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, 9’-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 (1H-1H, 13C–1H COSY), distortional enhancement by polarization transfer (DEPT), and heteronuclear multiple-bond connectivity (HMBC) experiments. The 1H-1H 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, 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 powder.

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der and their molecular formulas were determined by EI-MS and high-resolution EI-MS measurements to be \( \text{C}_{18}\text{H}_{20}\text{O}_{4} \), respectively. The UV spectra of 2 and 3 showed an absorption maximum \( [2, 262 \text{ nm} (\log \varepsilon 4.33); 3, 262 (4.35)] \), while their IR spectra showed absorption bands due to hydroxyl and olefin functions and aromatic ring \( (2, 3649, 1563, 1559, 1509, 1458 \text{ cm}^{-1}; 3, 3649, 1653, 1559, 1507, 1458 \text{ cm}^{-1}) \).

The proton and carbon signals in the \(^1\text{H}-\) and \(^{13}\text{C}-\text{NMR} \) (CD\(_3\)OD, Table 1) of 2 showed signals assignable to a methylene and a methine \( [\delta 1.92 \text{ (1H, m, 8-H)}, 2.23, 2.33 \text{ (1H each, both m, 9'-H\text{$_2$})}], a methylene and a methine bearing an oxygen function \( [\delta 3.40 \text{ (1H, ddd, } J=5.3, 10.9 \text{ Hz}), 3.56 \text{ (1H, dd, } J=6.0, 10.9 \text{ Hz), 9-H\text{$_2$})}], 4.74 \text{ (1H, d, } J=5.9 \text{ Hz, 7'-H)}, a \text{trans-olefin pair} \{\delta 6.00 \text{ (1H, ddd, } J=6.9, 7.7, 16.0 \text{ Hz, 8'-H}), 6.28 \text{ (1H, d, } J=16.0 \text{ Hz, 7'-H})\}, \) and two \text{para-}

<table>
<thead>
<tr>
<th>\text{Para-Substituted Aromatic Rings}</th>
<th>\text{CD}(_3)OD</th>
<th>\text{Pyridine-d}_5</th>
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</table>
| \( 6.68, 7.14 \text{ (2H each, both d, } J=8.6 \text{ Hz, 3',5'-H, 2',6'-H-H)} \) | 6.76, 7.18 \text{ (2H each, both d, } J=8.6 \text{ Hz, 3,5-H, 2,6-H-H)} \)

In addition, the proton and carbon signals in the \(^1\text{H}-\) and \(^{13}\text{C}-\text{NMR} \) (Table 1) spectra of 3 were similar to those of 2 \{a methylene and a methine \( [\delta 1.92 \text{ (1H, m, 8-H)}, 2.01, 2.10 \text{ (1H each, both m, 9'-H\text{$_2$})}], a methylene and a methine bearing an oxygen function \( [\delta 3.65 \text{ (1H, ddd, } J=5.3, 10.9 \text{ Hz}, 3,5'-H, 2',6'-H)} \)\}.
and 9-H, the molecular formula C\textsubscript{27}H\textsubscript{28}O\textsubscript{5} of the following proton and carbon pairs of experiment, long-range correlations were observed between the following proton and carbon pairs of 2 and 3 (2,6-H, 4,7-H; 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-acetone derivatives (2a, 3a). Treatment of 2 or 3 with 2,2-dimethoxypropane gave the 7,8-acetone derivatives (2a or 3a), respectively. As shown in Fig. 2, the coupling constants in the \(^1\)H-NMR spectrum of 2a showed \(\delta\) 1.38, 2.82 (2H each, both d, \(J=8.4\text{ Hz}\), 3′,5′-H; 2′,6′-H), 6.77, 7.18 (2H each, both d, \(J=8.4\text{ Hz}\), 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-H; 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.

\textbf{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 \(\epsilon\) 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\textsubscript{22}H\textsubscript{24}O\textsubscript{4} 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 (\(\delta\) 1.45 (1H, ddd, \(J=9.9, 13.4, 13.7\text{ Hz}\), 9\textsubscript{ax}-H), [1.82 (1H, ddd, \(J=7.5, 7.6, 14.0\text{ Hz}\), 2.14 (1H, m), 9\textsubscript{eq}-H], 1.89 (1H, br d, \(J=ca\). 13, 14 Hz, 9\textsubscript{eq}-H)], 2.14, 2.30 (1H each, both m, 8\textsubscript{eq}-H, 8\textsubscript{ax}-H), a methylene and two methines bearing an oxygen function (\(\delta\) 3.86 (1H, dd, \(J=2.5, 11.3\text{ Hz}\), 9\textsubscript{eq}-H), 4.19 (1H, d, \(J=9.7\text{ Hz}\), 7-H), 5.04 (1H, br d, \(J=ca\). 11 Hz, 9\textsubscript{eq}-H), 5.51 (1H, d, \(J=10.4\text{ Hz}\), 7\textsubscript{eq}-H)), a \textit{trans}-olefin pair (\(\delta\) 5.89 (1H, ddd, \(J=7.3, 7.6, 15.9\text{ Hz}\), 8\textsubscript{eq}-H), 6.26 (1H, d, \(J=15.9\text{ Hz}\), 7\textsubscript{eq}-H)), and three \(\textit{para}\)-substituted aromatic rings (\(\delta\) 7.15, 7.32 (2H each, both d, \(J=8.6\text{ Hz}\), 3′,5′-H, 2′,6′-H), 7.20, 7.61 (2H each, both d, \(J=8.6\text{ Hz}\), 3′,5′-H, 2′,6′-H), 7.24, 7.56 (2H each, both d, \(J=8.6\text{ Hz}\), 3,5-H, 2,6-H)). The \(^1\)H–\(^1\)H COSY and the HMBC experiments on 4 indicated the presence of \(^1\)H–\(^1\)H and \(^1\)H–\(^1\)C long range correlations, as shown in Fig. 1. The stereostructure of the \(\textit{trans}\)tetrahydropryan moiety in 4 was determined in a NOESY experiment, in which NOE correlations were observed between 7-H and 9\textsubscript{ax}-H, 9\textsubscript{ax}-H; 8-H and 9\textsubscript{eq}-H, 8\textsubscript{eq}-H and 9-H\textsubscript{z}, 9-H\textsubscript{z}, as shown in Fig. 1. This evidence was also suggested by the coupling constants in the \(^1\)H-NMR spectrum on 4 (\(\delta\) 7.8, 9.7 Hz and \(\delta\) 8.3, 2.5 Hz). On the basis of these findings, the structure of 4 was determined to be as shown.\(^{39}\)

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 sesquiole.

\(0.9\)-dimethoxypropane, Dowex HCR-W2 (H\(^+\) form), r.t., 12h

\textbf{Inhibitory Effects on NO Production in LPS-Activated Mouse Peritoneal Macrophages} The inorganic free radical NO has been implicated in pathologic and physiologic processes, such as vasodilatation, 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,\(^{37}\) triterpenes,\(^{38,41}\) diarylheptanoids,\(^{42,43}\) cyclic peptides, and alkaloids.\(^{44,45}\)

In the previous study, 1′S-1′-acetoxychavicol acetate (5) from \(A.\) \textit{galanga} (syn. \textit{Languas galanga}) was reported to inhibit NO production strongly in a macrophage-like cell line, RAW264.7,\(^{46}\) while the effects of the other constituents were not reported. Therefore the effects of the constituents (1—14) from the rhizomes of \(A.\) \textit{galanga} on NO production in LPS-activated macrophages were examined. As shown in Table 2, 1′S-1′-acetoxychavicol acetate (5, \(IC_{50}=2.3\mu\text{M}\)) exhibited the strongest activity among the isolated constituents, in agreement with the previous report.\(^{49}\) However, not only 5 but also galanganal (1, \(IC_{50}=68\mu\text{M}\)), galanganol B (3, \(88\mu\text{M}\)) and C (4, \(33\mu\text{M}\), 1′S-1′-acetoxychavicol acetate (5, \(2.3\mu\text{M}\)), 1′S-1′-acetoxyugenol acetate (6, \(11\mu\text{M}\)), \textit{trans}-p-hydroxyximnaldehyde (10, \(ca.\) 20 \(\mu\text{M}\)), \textit{trans}-p-coumaryl alcohol (11, \(72\mu\text{M}\)), and \textit{trans}-p-coumaryl diacetate (13, 19 \(\mu\text{M}\)) 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 [\(\text{N}^G\)-monomethyl-L-arginine (l-NMMA)] or an inhibitor of nuclear factor-κB activation [cafeic acid phenethyl ester (CAPE)].\(^{47}\) In addition, the structure–activity relationships of 5 in the inhibitory effects on NO production was described in our report.\(^ {45}\)
Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu-1600 spectrometer; IR spectra, Shimadzu FT-IR-8100 spectrometer; GC-MS and HPLC-MS, JMS-HG200, JMS-HG201 mass spectrometer; 1H-NMR spectra, JNM-LA500 (500 MHz) spectrometer; 13C-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., 100—250 mesh); reverse-phase silica gel column chromatography, Chromatorex ODS DM1020T (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 60F254 (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F254S (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with silica gel RP-18 WFS254 (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO4)2·10H2O aqueous solution followed by heating.

Plant Material

The rhizome of *A. galanga* was collected in Thailand in July 2001, and was identified by Dr. Yutana Pornpipatjida (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 [30 g, n-hexane-ethyl acetate (10:1—5:1; v/v)] gave 13 fractions [fr. 1 (0.7 g), 2 (2.3 g), 3 (0.5 g), 4 (2.8 g), 5 (1-hydroxychavicol acetate (5, 23.0 g, 11.0%), 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-H2O (70:30→90:10, v/v)] to give methylchavicol (9, 14 mg, 0.0006%), Fraction 6 (350 mg) was separated by preparative HPLC [CH3CN-H2O (40:60, v/v)] to give trans-p-coumaroyl diacetate (13, 63 mg, 0.015%) and 1′-o-hydroxychavicol acetate (6, 131 mg, 0.032%). Fraction 7 (2.6 g) was separated by reverse-phase silica gel column chromatography [80 g, MeOH-H2O (30:70→50:50, v/v)] to give p-hydroxybenzaldehyde (14, 99 mg, 0.0047%), 1′-o-hydroxychavicol acetate (7, 1005 mg, 0.008%), 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-H2O (25:75→50:50, v/v)] to give trans-p-coumaryl alcohol (11, 1090 mg, 0.052%) and galangal (1, 100 mg, 0.0048%). Fraction 11 (5.0 g) was subjected to reverse-phase silica gel column chromatography [150 g, MeOH-H2O (40:60→50:50→60:40→70:30→80:20, v/v)] to MeOH to furnish seven fractions [fr. 11-1 (824 mg), 11-2 (696 mg), 11-3 (379 mg), 11-4 (1169 mg), 11-5 (191 mg), 11-6 (551 mg), 11-7 (666 mg)]. Fraction 11-2 (696 mg) was subjected to HPLC [MeOH-H2O (50:50, v/v)] to give galangal A (2, 24 mg, 0.0011%) and B (3, 21 mg, 0.0010%). Fraction 11-4 (1169 mg) was further purified by HPLC [MeOH-H2O (60:40, v/v)] to give galangal C (4, 32 mg, 0.0015%). Fraction 12 (6.3 g) was separated by reverse-phase silica gel column chromatography [200 g, MeOH (25:75→40:60→50:40→70:30→80:20, v/v)] to give chavicol (8, 476 mg, 0.023%).

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

<table>
<thead>
<tr>
<th>Constituent</th>
<th>IC50 (μM)</th>
<th>Inhibition (%)</th>
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<tbody>
<tr>
<td>0 μM</td>
<td>1 μM</td>
<td>3 μM</td>
</tr>
<tr>
<td>Galangal (1)</td>
<td>0.0±1.2</td>
<td>12.7±5.8*</td>
</tr>
<tr>
<td>Galangal A (2)</td>
<td>0.0±3.7</td>
<td>1.2±5.0</td>
</tr>
<tr>
<td>Galangal B (3)</td>
<td>0.0±2.9</td>
<td>1.2±4.4</td>
</tr>
<tr>
<td>Galangal C (4)</td>
<td>0.0±2.0</td>
<td>-4.4±4.1</td>
</tr>
</tbody>
</table>

1′-S‘-Acetoxycineole acetate (5) | 0.0±1.1 | 17.6±11.1** | 59.9±1.6** | 97.5±0.5**m | 2.3 |

1′-S‘-Acetoxycineole 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‘-Hydroxycineole 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±2.8 | -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±2.1 | 13.4±7.4 | 8.2±8.4 | -0.5±10.7 | 78.4±11.0** | 109.3±11.7** |

trans-p-Hydroxycinnamaldehyde (11) | 0.0±4.0 | 1.6±5.1 | 7.5±3.6 | 17.4±5.0** | 29.4±1.9** | 61.0±2.9** |

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±2.7 | -0.3±6.0 | -0.2±12.3 | 26.7±14.3 | 76.8±12.6** | 96.3±1.7** |

trans-p-Coumaryl diacetate (13) | 0.0±2.8 | 5.2±3.6 | 9.0±2.2 | 1.9±0.5 | 8.7±7.6 | 17.2±2.9* |

p-Hydroxybenzaldehyde (14) | 0.0±2.0 | 5.9±2.9 | 10.3±3.7 | 15.0±1.6** | 34.1±3.2** | 63.1±1.2** |

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** |

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.
Preparation of the Acetidone 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.

Bioassy. NO Production from LPS-Stimulated Macrophages Inhibitory effects on the NO production by mouse macrophages were evaluated using the method reported previously. 43 Briefly, peritoneal exudate cells were collected from the peritoneal cavities of male ddY mice, which had been intraperitoneally injected with 4% thiglycolate medium 4 d before, and washed with 6–7 ml of ice-cold phosphate-buffered saline (PBS). The cells (5 × 10⁶ 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% CO₂ 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 cul-
ture supernatant. Cytotoxicity was determined using the 3-(4,5-dimethylthi-azolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay. Briefly, after 20-h incubation with test compounds, MTT (5 mg/ml in PBS) solution was added to the medium (final DMSO concentration 0.5%). Inhibition (%) was calculated using the following formula:

\[
\text{inhibition} \% = \frac{A - B}{A} \times 100
\]

\( A \) — C: NO₂⁻ concentration (μM): \( A \) : LPS (+); sample (−); \( B \) : LPS (+), sample (+); \( C \) : LPS (−), sample (−).

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

Acknowledgments This work was supported by the 21st COE and Academic Frontier Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

14) Methylenglycol (9) and p-hydroxybenzaldehyde (14) were identified by comparison of their physical data with those of commercial samples.
19) Galanganols A—C (2—4) were isolated as the racemic mixture, respectively, which were detected by HPLC [column: Shiseido Chiral CD-Ph (250×4.6 mm i.d.); detection: UV (254 nm); mobile phase: CH₃CN–H₂O (2 : 3)], 25.7, 26.4 min (ca. 1 : 1); 3, 23.2, 23.9 min (ca. 1 : 1); and 4, 19.8, 20.3 min (ca. 1 : 1).
20) The plane structure of galanganols A (2) and B (3) is identical with that proposed for (4E)-1,5-bis(4-hydroxyphenyl)-2-(4-hydroxymethyl)-4-penten-1-ol, which was isolated from Alpinia officinarum. 8
34) Muraoaka O., Fujimoto M., Tanabe G., Kubo M., Minematsu T., Ma-


