Medicinal Foodstuffs. XXVIII.[1] Inhibitors of Nitric Oxide Production and New Sesquiterpenes, Zedoarofuran, 4-Epicurcumenol, Neocurcumenol, Gajutsulactones A and B, and Zedoarolides A and B, from Zedoariae Rhizoma

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A new eudesmane-type sesquiterpene, zedoarofuran, and six new guaiane- or *seco*-guaiane-type sesquiterpenes, 4-epicurcumenol, neocurcumenol, gajutsulactones A and B, and zedoarolides A and B, were isolated from aqueous acetone extract of Zedoariae Rhizoma together with 36 known sesquiterpenes and two diarylheptanoids. Their stereostructures were elucidated on the basis of chemical and physicochemical evidence. The effects of isolated components on nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages were examined and 16 sesquiterpenes including gajutsulactones A and B, and bis(4-hydroxycinnamoyl)methane were found to show inhibitory activity.

Key words zedoarofuran; 4-epicurcumenol; neocurcumenol; gajutsulactone; zedoarolide; Zedoariae Rhizoma

Zedoary (*Curcuma zedoaria* ROSCOE, Zingiberaceae) has been extensively cultivated as a vegetable, spice, and perfume in South and Southeast Asian countries. The rhizome of *C. zedoaria* (Zedoariae Rhizoma) is widely used as a stimulant, stomachic, carminative, diuretic, anti-diarrheal, antiemetic, anti-pyretic, and depurator, and also to clean and cure ulcers, wounds, and other skin disorders in India and Southeast Asian countries. In Japanese and Chinese traditional medicines, Zedoariae Rhizoma (Japanese name, "Gajutsu"), which is listed in the Japanese Pharmacopoeia XIV, has been known to exhibit stomachic and emmenagogue-like effects. In particular, this natural medicine is prescribed in various Chinese preparations used for the treatment of "Oketsu" syndrome, which is thought to be caused by blood stagnation.

In the course of characterization studies on the bioactive constituents of Chinese natural medicines,[2] and medicinal foodstuffs,[1,3] we have already reported that the sesquiterpene constituents from Zedoariae Rhizoma exhibited potent vasorelaxant[4] and hepatoprotective activities.[5] In addition, we have characterized the absolute stereostructures of five carabrane-type sesquiterpenes called curcumenolactones A (**10**), B (**11**), and C (**12**), curcumenone (**13**), 4*S*-dihydrocurcumenone (**14**), and curcarabranols A (**15**) and B (**16**) on the basis of chemical and physicochemical evidence.[4,5] As a continuation of the characterization studies of Zedoariae Rhizoma, we have isolated a eudesmane-type sesquiterpene called zedoarofuran (**1**), and two guaiane- and two *seco*-guaiane-type sesquiterpenes, 4-epicurcumenol (**2**), neocurcumenol (**3**), and gajutsulactones A (**4**) and B (**5**), from the ethyl acetate-soluble portion. Furthermore, two guaiane-type sesquiterpenes called zedoarolides A (**6**) and B (**7**) were also isolated from the 1-butanol-soluble portion. This paper deals with the isolation and structural elucidation of new sesquiterpenes (**1**—**7**) from Zedoariae Rhizoma. In addition, we describe the inhibitory effect of the components on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.[6]

The 80% aqueous acetone extract of Zedoariae Rhizoma (cultivated in Szechwan province, China) was partitioned into a mixture of ethyl acetate and water to furnish the ethyl acetate-soluble portion and an aqueous phase. The aqueous phase was further extracted with 1-butanol to give a 1-butanol-soluble portion and water-soluble portion as previously described.[5] The ethyl acetate-soluble portion was subjected to silica gel and octadecyl silica gel (ODS) column chromatography and finally HPLC to furnish zedoarofuran (**1**, 0.0002% from natural medicine), 4-epicurcumenol (**2**, 0.0006%), neocurcumenol (**3**, 0.0014%), gajutsulactones A (**4**, 0.0002%) and B (**5**, 0.0002%), furanodienone (**8**,[7] 0.0002%), and curcolone (**9**,[8] 0.0006%) together with 30 sesquiterpenes (**10**—**33**, **37**, **38**, **40**—**43**) and a diarylheptanoid, curcumin (**44**). The 1-butanol-soluble portion was also separated by the above chromatography to give zedoarolides A (**6**, 0.0002%) and B (**7**, 0.0007%) together with five sesquiterpenes (**33**—**36**, **39**) and a diarylheptanoid bis(4-hydroxycinnamoyl)methane (**45**).

Stereostructure of Zedoarofuran (1) Zedoarofuran (**1**) was isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{24}$ +26.0°, $CHCl_3$) and was deduced to possess a furan ring based on TLC examination using Ehrlich's reagent. The electron impact (EI)-MS of **1** showed a molecular ion (M^+) peak at *m*/*z* 264 in addition to fragment ion peaks at *m*/*z* 246 (M^+–H_2O) and *m*/*z* 163 (base peak). The molecular formula $C_{15}H_{20}O_4$ of **1** was determined from the molecular ion peak observed in the EI-MS and by high-resolution MS measurement. The IR spectrum of **1** showed absorption bands at 3456, 1724, and 887 cm^{-1} ascribable to the hydroxyl and carbonyl functions and furan ring, respectively. In the UV spectrum of **1**, an absorption maximum was observed at 266 nm (log ε 3.33), suggestive of a conjugated carbonyl group. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1** showed signals assignable to three methyls [δ 1.15, 1.44 (both s, 14 and 15-H_3), 2.18 (d, J=1.2 Hz, 13-H_3)], a methine bearing a hydroxyl group [δ 3.62 (dd, J=2.6, 10.3 Hz, 1-H)], and a furan ring [δ 7.09 (d, J=1.2 Hz, 12-H); δ_C 118.7 (7-C), 165.3 (8-C), 120.4 (11-C), 139.5 (12-C)] together with three

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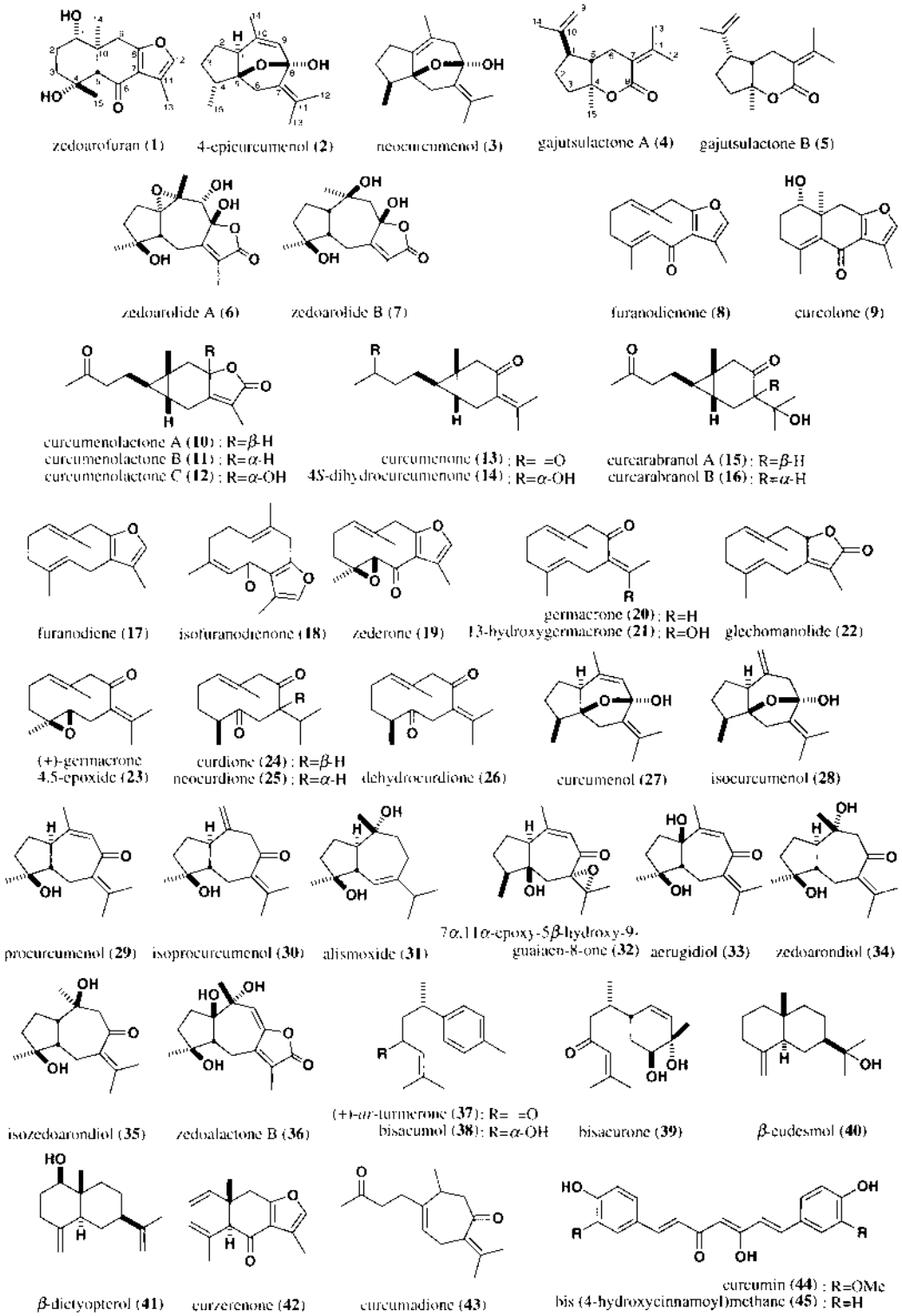

Chart 1

methylenes (2, 3, 9-H_2), a methine (5-H), and three quaternary carbons (4, 6, 10-C).

The planar structure of **1** was constructed on the basis of ^1H–^1H correlation spectroscopy (H–H COSY) and heteronuclear multiple bond correlation (HMBC) experiments. Thus the H–H COSY experiment on **1** indicated the presence of a partial structure written in bold line, as shown in Fig. 1. In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **1** (3, 5-H, 15-H_3 and 4-C; 5-H and 6, 10-C; 13-H_3 and 7, 11, 12-C; 9-H_2 and 8, 10-C; 14-H_3 and 1, 10-C), so that the connectivities of the quaternary carbons (4, 6, 10-C) and furan ring (7, 8, 11, 12-C) in **1** were clarified. The above-mentioned evidence led us to confirm the skeleton of zedoarofuran (**1**) to be 6-oxo-8,12-epoxy-7,11-eudesmadien-1,4-diol. Furthermore, the relative stereostructure of **1** was clarified by a nu-

Table 1. ^{13}C-NMR Data for Zedoarofuran (**1**), 4-Epicurcumenol (**2**), Neocurcumenol (**3**), Gajutsulactones A (**4**) and B (**5**), Zedoarolides A (**6**) and B (**7**), and Related Compounds (**3a**, **3b**, **6a**)

	1[a]	**2**[a]	**2**[b]	**3**[a]	**3a**[a]	**3b**[a]	**4**[a]	**5**[a]	**6**[b]	**6**[c]		**6a**[c]	**7**[b]	**7**[c]
C-1	78.4	48.8	48.9	137.6	49.2	49.3	47.5	42.6	70.3	71.5		70.5	53.2	53.0
C-2	26.8	28.3	28.8	23.4	21.7	21.4	26.7	26.3	29.6	29.8		29.9	25.3	25.6
C-3	38.3	31.6	32.1	29.8	29.9	30.3	36.7	38.2	39.5	39.1		36.6	38.3	38.0
C-4	69.8	41.5	42.3	39.4	38.9	39.3	85.2	85.3	78.7	79.8		88.6	80.8	82.3
C-5	59.8	87.7	87.1	86.4	86.9	87.5	45.7	45.9	50.8	50.5		49.8	52.4	52.3
C-6	198.0	37.5	38.1	44.3	35.9	30.2	26.9	25.8	20.9	20.8		21.1	24.6	24.8
C-7	118.7	122.2	138.8	126.5	126.0	48.0	119.7	120.5	159.0	158.9		156.3	161.5	162.0
C-8	165.3	101.3	101.8	104.1	103.7	103.9	167.2	167.4	109.3	109.1		115.2	106.9	107.2
C-9	39.1	125.9	128.9	42.4	39.7	43.0	110.5	111.9	77.6	77.8		75.7	44.1	43.9
C-10	45.6	138.4	137.1	123.5	28.2	26.7	145.8	145.3	62.3	62.8		61.0	72.2	73.4
C-11	120.4	137.1	120.7	136.2	134.8	27.3	152.1	151.6	125.7	127.1		128.8	122.8	124.1
C-12	139.5	19.0[d]	19.3[d]	19.4[d]	19.0[d]	18.2[d]	23.3[d]	23.3[d]	173.0	174.3		172.0	173.7	175.3
C-13	8.8	22.2[d]	22.3[d]	22.6[d]	22.5[d]	22.3[d]	23.6[d]	23.5[d]	8.3	8.2		8.3	8.0	7.9
C-14	12.7	21.1	21.2	18.3	18.5	18.6	19.5	25.1	22.1	21.8		21.9	32.5	31.7
C-15	31.2	16.8	16.9	15.0	12.4	12.6	19.5	20.0	22.5	21.8		18.9	25.6	24.9
											$\underline{C}H_3CO$-	20.3		
												21.4		
												23.8		
											$\underline{C}H_3CO$-	169.6		
												170.5		
												172.0		

Measured in *a*) $CDCl_3$, *b*) C_5D_5N, and *c*) CD_3OD at 125 MHz. *d*) May be interchangeable within the same column.

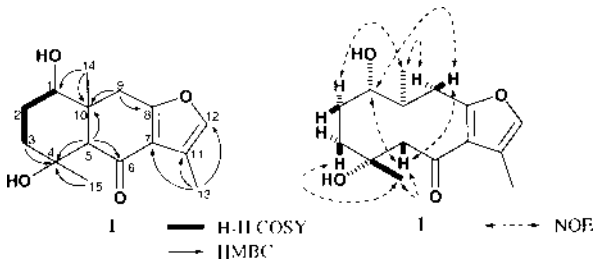

Fig. 1

clear Overhauser effect spectroscopy (NOESY) experiment, which showed the NOE correlations between the following proton pairs: 1-H and 5-H, 9β-H; 2α-H and 14-H_3; 3β-H and 15-H_3; 5-H and 9β-H, 15-H_3; 9α-H and 14-H_3 (Fig. 1). Consequently, the stereostructure of zedoarofuran (**1**) was determined to be as shown.

Stereostructures of 4-Epicurcumenol (2), and Neocurcumenol (3) 4-Epicurcumenol (**2**) was isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{26}$ +120.1°, $CHCl_3$). The molecular formula $C_{15}H_{22}O_2$ of **2** has been determined from the molecular ion peak at *m/z* 234 (M^+) in the EI-MS and by high-resolution MS measurement. The IR spectrum of **2** showed absorption bands at 3370, 1694, 1663, and 1065 cm^{-1} ascribable to the hydroxyl, olefin, and ether functions. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **2** showed signals assignable to a secondary methyl [δ 0.95 (d, J=6.9 Hz, 15-H_3)], three tertiary methyls [δ 1.59, 1.80 (both s, 12, 13-H_3), 1.67 (s, 14-H_3)], a trisubstituted olefin [δ 5.78 (br s, 9-H), δ_C 125.9 (9-C), 138.4 (10-C)], and tetrasubstituted olefin [δ_C 122.2 (7-C), 137.1 (11-C)] together with three methylenes (2, 3, 6-H_2), two methines (1, 4-H), and two quaternary carbons (5, 8-C). The partial structure of **2** written in bold in Fig. 2 was clarified by H–H COSY and long-range correlations were observed between the following protons and carbons of **2** (1-H, 4-H, 6-H_2 and 5-C; 6-H_2 and 7-C; 9-H and 8-C; 1-H, 14-H_3 and 10-C) in the HMBC experiment (Fig. 2). The proton and carbon signals in the NMR spectra of **2** were superimposable on those of curcumenol (**27**),[9] which was a principal guaiane-type sesquiterpene from Zedoaria Rhizoma, except for the signals due to the 4 and 15-carbons. In addition, the NOE correlations of **2** were observed between the following proton pairs: 1-H and 2α-H, 6α-H, 15-H_3; 2α-H and 3α-H; 2β-H and 3β-H; 3α-H and 15-H_3; 4-H and 6β-H; 6α-H, 6β-H and 15-H_3, while **27** showed following pairs: 1-H and 4-H, 6α-H; 3β-H and 15-H_3; 4-H and 6α-H, 6β-H; 6β-H and 15-H_3, as shown in Fig. 2. On the basis of the above-mentioned evidence, the stereostructure of **2** was clarified as shown.

Neocurcumenol (**3**) was also isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{25}$ +15.3°, $CHCl_3$). The IR spectrum of **3** showed absorption bands due to the hydroxyl, olefin, and ether functions at 3379, 1694, and 1051 cm^{-1}. The EI-MS of **3** showed a molecular ion peak at *m/z* 234 (M^+) and 105 (base peak) and the molecular formula $C_{15}H_{22}O_2$, which is the same as that of **2** or **27**, was determined by high-resolution MS measurement. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR (Table 1) spectra of **3** were also found to be similar to those of **2** or **27** and indicated the presence of similar functional groups except for lacking an olefin proton. That is, the ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **3** showed signals due to a secondary methyl [δ 0.95 (d, J=7.0 Hz, 15-H_3)], three methyls [δ 1.56 (s, 14-H_3), 1.60, 1.84 (both s, 12, 13-H_3)], and two tetrasubstituted olefins (1, 7, 10, 11-C) together with four methylenes (2, 3, 6, 9-H_2), a methine (4-H), and two quaternary carbons (5, 8-C). In addition, the connectivities of the ^{1}H–^{1}H and quaternary carbons in **3** was clarified by H–H COSY and HMBC experiments, as shown in Fig. 2. In addition, the relative stereostructure of **3** was characterized by NOESY experiments, which NOE correlations were observed between the signals of the following proton pairs: 2α-

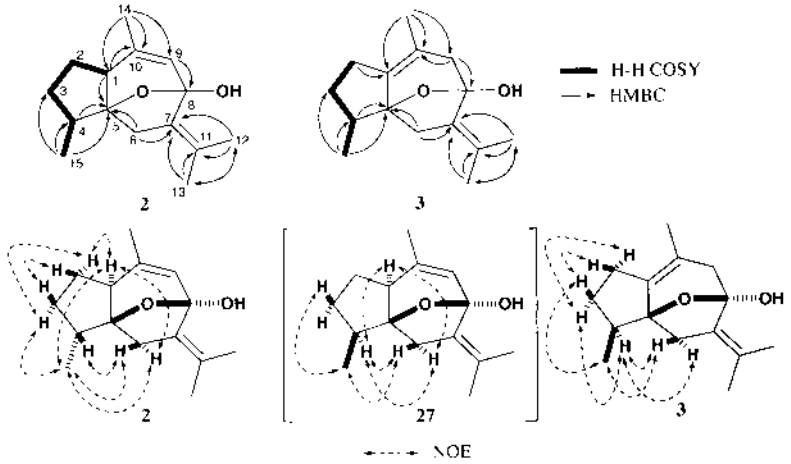

Fig. 2

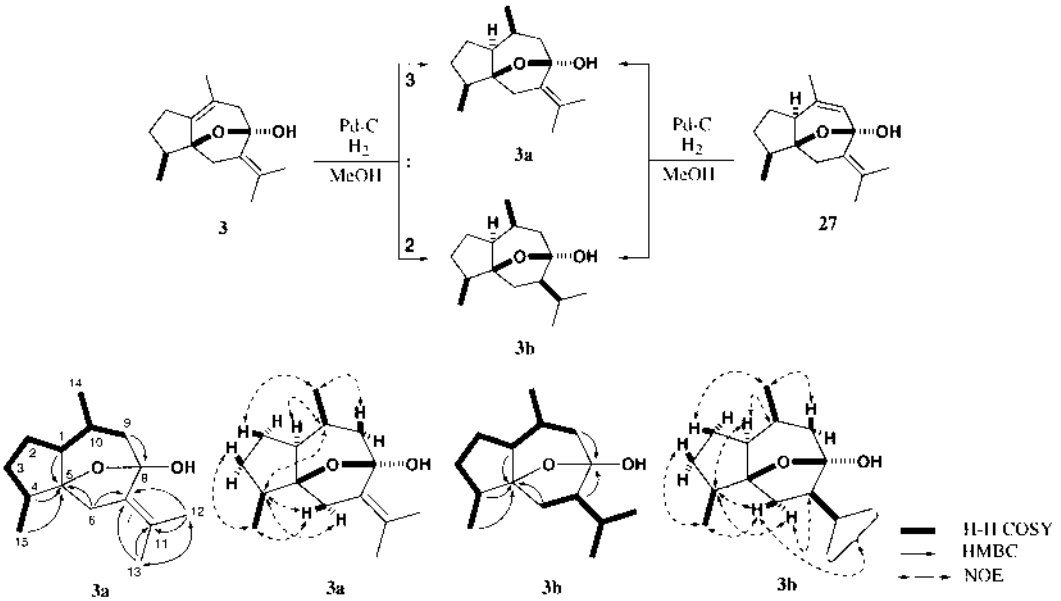

Fig. 3

H and 3α-H; 2β-H and 3β-H; 3α-H and 4-H; 3β-H and 15-H_3; 4-H and 6α-H, 6β-H; 6β-H and 15-H_3.

In order to elucidate the absolute stereostructure, **3** was chemically related to **27**, for which the absolute configuration has been reported.[9] Thus hydrogenation of **3** in the presence of 10% Pd–C in MeOH furnished the 1α,10α-dihydro-derivative (**3a**) and the 1α,7α,10α,11α-tetrahydro-derivative (**3b**) in a 3 : 2 ratio. The stereostructures of **3a** and **3b** were determined by H–H COSY, HMBC, and NOESY experiments, as shown in Fig. 3. On the other hand, **3a** and **3b** were also obtained by hydrogenation of **27**, and thus the absolute stereostructure of neocurcumenol was determined to be the $\Delta^{1(10)}$-isomer (**3**) of curcumenol (**27**).

Stereostructures of Gajutsulactones A (4) and B (5) Gajutsulactones A (**4**) and B (**5**) were isolated as colorless oil with negative optical rotation (**4**: $[\alpha]_D^{28}$ $-128.4°$; **5**: $[\alpha]_D^{27}$ $-35.0°$ in $CHCl_3$). The EI-MS of **4** and **5** showed the same molecular ion peak at *m/z* 234 (M^+) together with a fragment ion peak at *m/z* 107 (base peak), and the molecular formula $C_{15}H_{22}O_2$ was determined by high-resolution MS measurement. In the UV spectra (MeOH) of **4** and **5**, absorption maxima were observed at 233 nm (**4**, log ε 4.03) and 232 nm (**5**, log ε 4.37), suggestive of an α,β-unsaturated lactone moiety. The IR spectra of **4** and **5** showed absorption bands due to the olefin, α,β-unsaturated carbonyl, and ether functions (**4**: 1700, 1615, 1061 cm^{-1}; **5**: 1713, 1620, 1067 cm^{-1}). The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **4** showed the presence of four methyls {δ 1.23 (s, 15-H_3), 1.72 (d, J=0.6 Hz, 14-H_3), [1.84 (s), 2.22 (dd, J=1.6, 1.8 Hz), 12, 13-H_3]}, an *exo*-methylene [δ 4.74, 4.76 (1H each, both d like, 9-H_2), δ_C 110.5 (9-C), 145.8 (10-C)], and a tetrasubstituted olefin [δ_C 119.7 (7-C), 152.1 (11-C)] together with three methylenes (2, 3, 6-H_2), two methines (1, 5-H), and two quaternary carbons (4, 8-C). The proton signals in the ^{1}H-NMR ($CDCl_3$) spectrum of **5** were found to be superimposable on those of **4** and indicated the presence of the same functional groups. The planar structures of **4** and **5**, which were identified with unique *seco*-guaiane-type skeleton first proposed for caulolactones A and B from *Asarum caulescens*,[10] were constructed on the basis of H–H COSY,

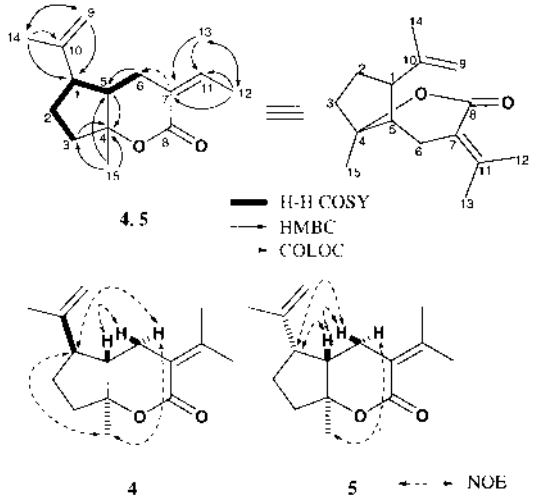

Fig. 4

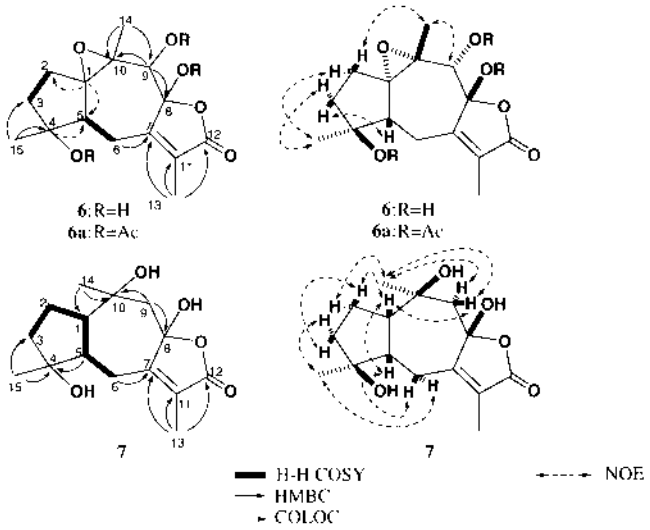

Fig. 5

HMBC, and correlation *via* long-range coupling (COLOC) experiments. Thus the H–H COSY experiments on **4** and **5** indicated the presence of a partial structure shown in bold in Fig. 4: from C-1—C-3 and from C-1—C-6. In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **4** and **5**: 3-H_2, 5-H, 15-H_3 and 4-C; 12-H_3 and 7, 11, 13-C; 13-H_3 and 7, 11, 12-C; 9-H_2, 14-H_3 and 1-C, and the COLOC experiment showed a correlation between the 7-C and the 6-H. That evidence led us to confirm the skeleton of **4** and **5** to be 8,9-*seco*-7(11),9(10)-guaiadien-8,4-olide.

The stereostructures of **4** and **5** were elucidated on the basis of NOESY spectra, in which the NOE correlations of **4** were observed between the 1-proton and the 6α, 15-protons and between the 5-proton and the 6β-proton, while the NOE correlation of **5** were observed between the 1-proton and the 5, 6β-protons, between the 5-proton and the 6β-proton, and between the 6α-proton and the 15-protons, as shown in Fig. 4. Thus the stereostructures of **4** and **5** were elucidated and they were determined to be the stereoisomers at the 1-position.

Stereostructures of Zedoarolides A (6) and B (7) Zedoarolide A (**6**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{18}$ −32.5°, MeOH). The molecular formula $C_{15}H_{20}O_6$ of **6** has been determined from the quasimolecular ion peak at *m/z* 297 $(M+H)^+$ and *m/z* 295 $(M-H)^-$ in the positive- or negative-ion fast atom bombardment (FAB)-MS and by high-resolution MS measurement. The IR spectrum of **6** showed absorption bands at 3453, 1736, 1686, and 1062 cm^{-1} ascribable to the hydroxyl, α,β-unsaturated γ-lactone, olefin, and ether functions, while its UV spectrum [absorption maximum: 217 nm (log ε 3.81) in MeOH] also indicated the presence of an α,β-unsaturated γ-lactone chromophore. The ^{1}H-NMR (C_5D_5N) and ^{13}C-NMR (Table 1) spectra of **6** showed signals assignable to three methyls [δ 1.52, 1.63, 1.80 (both s, 14, 15, 13-H_3)], a methine bearing an oxygen function [δ 4.84 (s, 9-H)], and a tetrasubstituted olefin [δ_C 159.0 (7-C), 125.7 (11-C)] together with three methylenes (2, 3, 6-H_2), a methine (5-H), and five quaternary carbons (1, 4, 8, 10, 12-C). To clarify the number of hydroxyl groups, acetylation of **6** using acetic anhydride (Ac_2O)–pyridine in the presence of 4-dimethylaminopyridine (4-DMAP) furnished the triacetate (**6a**). The ^{1}H-NMR (CD_3OD) spectrum of **6a** showed the presence of three acetyl groups [δ 1.98, 2.04, 2.11 (both s, –OAc)] and a methine bearing an acetoxyl group [δ 4.84 (br s)]. Comparison of the ^{13}C-NMR (Table 1) data from **6a** with those of **6** revealed acetylation shifts around the 4, 8, and 9-positions. The H–H COSY experiment of **6** and **6a** showed two partial structures in bold in Fig. 5 and the HMBC and COLOC experiments showed long-range correlations between the following protons and carbons: [HMBC (6-H and 7-C; 9-H and 8, 10-C; 13-H_3 and 7, 11, 12-C; 14-H_3 and 9, 10-C; 15-H_3 and 3, 4-C), COLOC (1-C and 2, 5-H; 4-C and 5-H)]. Thus the plane structure of **6** was characterized as 1,10-epoxy-4,8,9-trihydroxy-7(11)-guaien-12,8-olide.

The NOESY experiment on **6** showed NOE correlations between the signals of following proton pairs: 2α-H and 15-H_3; 2β-H and 14-H_3; 3α-H and 15-H_3; 3β-H and 5-H; 9-H and 14-H_3, as depicted in Fig. 5. On the basis of these findings, the stereostructure of **6** was elucidated, except for the 8-position.

Zedoarolide B (**7**) was also isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{21}$ −20.6°, MeOH). The positive-ion and negative-ion FAB-MS of **7** showed a quasimolecular ion peak at *m/z* 283 $(M+H)^+$ and 281 $(M-H)^-$, respectively and the molecular formula $C_{15}H_{22}O_5$ of **7** was determined by high-resolution MS measurement. In the UV spectrum of **7**, an absorption maximum was observed at 223 nm (log ε 3.82 in MeOH), suggestive of an α,β-unsaturated γ-lactone function. The IR spectrum of **7** showed absorption bands at 3475, 1719, 1686, and 1000 cm^{-1} ascribable to the hydroxyl, α,β-unsaturated γ-lactone, olefin, and ether functions. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **7** resembled those of **6**, except for the signals due to the 1, 9, and 10 positions. The ^{1}H-NMR (C_5D_5N) and ^{13}C-NMR (Table 1) spectra of **7** indicated the presence of three methyls [δ 1.43, 1.57, 1.80 (both s, 14, 15, 13-H_3)] and a tetrasubstituted olefin [δ_C 161.5 (7-C), 122.8 (11-C)] together with four methylenes (2, 3, 6, 9-H_2), two methines (1, 5-H), and four quaternary carbons (4, 8, 10, 12-C). The H–H COSY experiment on **7** indicated a partial

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

	Inhibition (%)		
	10 μM	30 μM	100 μM
Sesquiterpenes			
1) Carabrane type			
Curcumenolactone A (**10**)	−11.3±2.8	−0.8±0.7	40.2±3.2**
Curcumenolactone B (**11**)	−14.1±3.4*	−1.9±2.3	30.6±4.7**
Curcumenolactone C (**12**)	−16.4±5.2	−5.5±5.0	−1.4±4.3
Curcumenone (**13**)	10.4±2.1*	27.9±1.7**	54.8±1.4**
4*S*-Dihydrocurcumenone (**14**)	−9.6±1.8*	−4.5±1.1	13.0±2.0**
Curcarabranol A (**15**)	8.6±3.0	19.2±1.9**	28.8±2.1**
Curcarabranol B (**16**)	18.9±3.3**	22.6±2.3**	35.1±1.0**
2) Germacrane type			
Furanodiene (**17**)	0.1±1.5	8.7±2.1	67.0±1.4**
Isofuranodienone (**18**)	−7.9±2.9	22.1±3.3**	64.6±2.6**
Zederone (**19**)	2.0±2.8	9.3±0.7*	29.9±2.4**
Germacrone (**20**)	1.2±2.5	11.2±2.2*	32.7±1.3**
13-Hydroxygermacrone (**21**)	7.5±4.1	11.4±1.9	50.7±1.9**
Glechomanolide (**22**)	−1.0±2.9	37.2±4.4**	86.5±1.0**
(+)-Germacrone 4,5-epoxide (**23**)	−3.1±2.5	6.8±1.9	29.5±4.5**
Curdione (**24**)	4.6±3.5	16.9±3.8*	32.0±1.6**
Neocurdione (**25**)	1.8±2.6	21.3±2.9**	50.4±2.3**
Dehydrocurdione (**26**)	−4.8±3.7	5.5±2.5	12.8±3.1**
3) Guaiane type			
4-Epicurcumenol (**2**)	−2.8±3.0	10.1±0.9*	40.1±1.4**
Neocurcumenol (**3**)	−3.5±4.1	9.5±2.2	45.4±2.2**
Gajutsulactone A (**4**)	−9.2±6.4	5.5±6.3	53.6±3.0**
Gajutsulactone B (**5**)	−1.5±1.6	13.3±1.7*	57.5±3.5**
Zedoarolide A (**6**)	−14.1±0.8*	−12.7±2.3*	−3.9±4.3
Zedoarolide B (**7**)	−12.7±3.6*	−2.3±1.5	10.9±4.4
Curcumenol (**27**)	5.4±4.6	30.7±3.4**	71.3±2.1**
Isocurcumenol (**28**)	5.7±5.7	33.0±2.7**	65.8±2.8**
Procurcumenol (**29**)	−2.4±4.5	30.4±5.0**	67.8±4.4**
Isoprocurcumenol (**30**)	−5.7±7.6	10.4±3.4	20.6±2.9*
Alismoxide (**31**)	0.4±4.2	12.0±2.6*	33.1±4.1**
7α-11α-Epoxy-5β-hydroxy-9-guaiaen-8-one (**32**)	−9.7±1.5	14.8±1.3*	32.0±2.0**
Aerugidiol (**33**)	5.9±2.6	5.7±4.5	12.5±1.6
Zedoarondiol (**34**)	−9.5±4.1	−6.7±2.1	10.3±2.0**
Isozedoarondiol (**35**)	0.2±1.5	7.9±3.7	11.4±2.5*
Zedoalactone B (**36**)	−15.3±7.1	−7.4±4.4	−7.1±7.6
4) Bisaborane type			
(+)-*ar*-Turumerone (**37**)	−3.3±2.1	15.8±2.5**	52.9±2.8**
Bisacumol (**38**)	−4.9±2.2	15.7±0.2**	61.9±1.5**
Bisacurone (**39**)	10.2±2.6	23.2±1.9**	54.3±4.0**
5) Eudesmane type			
β-Eudesmol (**40**)	5.5±1.8	31.6±1.6**	98.5±1.8**
β-Dictyopterol (**41**)	−9.1±1.9	10.3±2.5*	51.5±3.5**
6) Elemane type			
Curzerenone (**42**)	12.4±1.8*	23.8±4.4**	39.7±2.4**
7) Xanthane type			
Curcumadione (**43**)	2.7±2.5	8.1±2.0	27.2±2.2**
Diarylheptanoides			
Curcumin (**44**)	35.6±2.2**	83.8±1.0**	103.0±0.7**[a]
Bis(4-hydroxycinnamoyl)methane (**45**)	2.9±0.9	7.4±2.3	57.1±3.4**
L-NMMA[b]	17.7±2.8**	52.3±1.5**	79.2±0.9**

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 (viability: 4%). *b*) L-NMMA: N^G-monomethyl-L-arginine.

structure shown in bold in Fig. 5. In the HMBC experiment on **7**, long-range correlations were observed between the following protons and carbons: 5-H, 15-H_3 and 4-C; 6-H and 7-C; 9-H and 8, 10-C; 13-H_3 and 7, 11, 12-C; 14-H_3 and 1, 10-C, 15-H_3 and 3-C. Furthermore, the relative stereostructure of **7** was characterized by NOESY experiments, in which NOE correlations were observed between the signals of the following proton pairs: 1-H and 2β-H, 5-H, 9β-H; 5-H and 6β-H; 14-H_3 and 2α-H, 9α-H, 9β-H; 15-H_3 and 3α-H, 6α-H, and therefore the stereostructure of **7** was characterized, except for the 8-position.

Finally, the stereostructures of the 8-hydroxyl groups in **6** and **7** were determined by the circular dichroic (CD) spectrum on α,β-unsaturated γ-lactone moiety.[5b,11] That is, the CD spectra of **6** and **7** showed the characteristic Cotton curve [**6**: $\Delta\varepsilon$ +5.15 (222 nm), −3.33 (245 nm), **7**: $\Delta\varepsilon$ +1.76 (226 nm), −3.64 (247 nm) in MeOH] in *endo*-α,β-unsatulated γ-lactones, and thus the absolute stereostructure of **6**

and **7** were determined to be 8*R* and 8*S* configurations, respectively.

Inhibitory Activity of Constituents from Zedoariae Rhizoma against NO Production in LPS-Activated Mouse Peritoneal Macrophages The inorganic free radical NO has been implicated in physiological and pathological 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 NOS family, inducible NOS in particular is involved in a pathological aspect with overproduction of NO, and can be expressed in response to pro-inflammatory agents such as interleukin-1β, tumor necrosis factor-α, and LPS in various cells including macrophages, endothelial cells, and smooth muscle cells.

As a part of our characterization studies on the bioactive components of natural medicines, we previously reported several NO production inhibitors: higher unsaturated fatty acids[12]; polyacetylenes[13,14]; coumarins[13]; flavonoids[14]; stilbenes[15]; lignans[16]; sesquiterpenes[14,17]; diterpenes[18]; and triterpenes.[19] In addition, we also previously reported that the inhibitory effect and mechanism of action of sesquiterpenes from Zedoariae Rhizoma on D-galactosamine/LPS-induced liver injury.[5] Continuous screening for the effect of isolated constituents from Zedoariae Rhizoma on NO production from LPS-activated macrophages was examined, and the results are summarized in Table 2. Gajustulactones A (**4**, IC_{50}=93 μM) and B (**5**, 84 μM), which were two new *seco*-guaiane-type sesquiterpenes, and 14 sesquiterpenes [curcumenone (**13**, 82 μM), furanodiene (**17**, 75 μM), isofuranodienone (**18**, 68 μM), 13-hydroxygermacrone (**21**, 98 μM), glechomanolide (**22**, 42 μM), neocurdione (**25**, 98 μM), curcumenol (**27**, 55 μM), isocurcumenol (**28**, 57 μM), procurcumenol (**29**, 56 μM), (+)-*ar*-turumerone (**37**, 92 μM), bisacumol (**38**, 76 μM), bisacurone (**39**, 86 μM), β-eudesmol (**40**, 44 μM),[17] and β-dictyopterol (**41**, 96 μM)], and two diarylheptanoids curcumin (**44**, 13 μM) and [bis(4-hydroxycinnamoyl)methane (**45**, 87 μM)] were found to inhibit NO production. The inhibitory activity of these components against NO production may be important evidence substantiating the traditional effects of this herbal medicine for the treatment of "Oketsu" syndrome caused by blood stagnation with inflammation.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, CD spectra, JASCO J-720WI; Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A 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.

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); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 or Ehrlich's reagent followed by heating.

Isolation of Zedoarofuran (1), 4-Epicurcumenol (2), Neocurcumenol (3), Gajutsulactones A (4) and B (5), Zedoarolides A (6) and B (7), Furanodienone (8), and Curcolone (9) from Zedoariae Rhizoma Zedoarofuran (**1**), 4-epicurcumenol (**2**), neocurcumenol (**3**), gajutsulactones A (**4**) and B (**5**), and zedoarolides A (**6**) and B (**7**) were isolated from Zedoariae Rhizoma cultivated in Szechwan province, China, as described earlier.[5b]

Fraction 1 (3.6 g) from the ethyl acetate-soluble, from which furanodiene (**17**) was isolated as reported previously,[5b] was further separated by silver nitrate-treated silica gel column chromatography [*n*-hexane–AcOEt (50 : 1→10 : 1)→AcOEt] to furnish furanodienone (**8**, 9 mg). In addition, fraction 10 (1.4 g), from which 4*S*-dihydrocurcumenone (**14**), 13-hydroxygermacrone (**21**), and curcumadione (**43**) were previously isolated,[5b] was subjected to HPLC [YMC-Pack ODS-A, MeOH–H_2O (60 : 40, v/v)] to furnish curcolone (**9**, 5 mg).

Zedoarofuran (**1**): Ehrlich's reagent positive. Colorless oil, $[\alpha]_D^{24}$ +26.0° (c=0.10, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{20}O_4$ (M^+): 264.1362. Found: 264.1357. UV [MeOH, nm, (log ε)]: 266 (3.33). IR (film): 3456, 2932, 1724, 1669, 1082, 887 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.15, 1.44 (3H each, both s, 14, 15-H_3), 1.61 (1H, m, 2β-H), 1.85, 1.88 (1H each, both m, 3-H_2), 1.94 (1H, m, 2α-H), 2.18 (3H, d, J=1.2 Hz, 13-H_3), 2.47 (1H, s, 5-H), 2.75, 3.01 (2H, ABq, J=17.4 Hz, 9-H_2), 3.62 (1H, dd, J=2.6, 10.3 Hz, 1-H), 7.09 (1H, d, J=1.2 Hz, 12-H). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 264 (M^+, 30), 246 (M^+–H_2O, 15), 163 (100).

4-Epicurcumenol (**2**): Colorless oil, $[\alpha]_D^{26}$ +120.1° (c=1.80, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{22}O_2$ (M)$^+$: 234.1620. Found: 234.1620. IR (film): 3370, 2962, 1694, 1663, 1065 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, d, J=6.9 Hz, 15-H_3), 1.30 (1H, m, 3α-H), 1.54 (1H, m, 2β-H), 1.59, 1.80 (3H each, both s, 12, 13-H_3), 1.67 (3H, s, 14-H_3), 1.94 (2H, m, 1, 2α-H), 2.15 (2H, m, 3β, 4-H), 2.18, 2.66 (1H each, both d, J=15.2 Hz, 6β, 6α-H), 3.38 (1H, br s, 8-OH), 5.78 (1H, br s, 9-H). ^{1}H-NMR (C_5D_5N) δ: 0.92 (3H, d, J=7.1 Hz, 15-H_3), 1.30 (1H, m, 3α-H), 1.54, 2.04 (3H each, both s, 12, 13-H_3), 1.60 (1H, m, 2β-H), 1.64 (3H, s, 14-H_3), 1.99 (1H, m, 2α-H), 2.05 (1H, m, 1-H), 2.23 (2H, m, 3β, 4-H), 2.32 (1H, d, J=15.6 Hz, 6β-H), 2.74 (1H, dd, J=1.5, 15.6 Hz, 6α-H), 4.91 (1H, br s, 8-OH), 6.17 (1H, br s, 9-H). ^{13}C-NMR ($CDCl_3$, C_5D_5N) δ_C: given in Table 1. EI-MS m/z (%): 234 (M^+, 30), 105 (100).

Neocurcumenol (**3**): Colorless oil, $[\alpha]_D^{25}$ +15.3° (c=2.00, $CHCl_3$). High-resolution EI-MS Calcd for $C_{15}H_{22}O_2$ (M^+): 234.1620. Found: 234.1623. IR (film): 3379, 2915, 1694, 1051 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, d, J=7.0 Hz, 15-H_3), 1.48 (1H, dddd, J=2.1, 2.1, 6.7, 12.8 Hz, 3β-H), 1.56 (3H, s, 14-H_3), 1.60, 1.84 (3H each, both s, 12, 13-H_3), 1.84 (1H, m, 3α-H), 2.08 (1H, dq, J=6.4, 7.0 Hz, 4-H), 2.13 (1H, m, 2α-H), 2.19, 2.47 (2H, ABq, J=17.0 Hz, 9-H_2), 2.34 (1H, m, 2β-H), 2.34 (1H, d like, J=*ca.* 14 Hz, 6β-H), 2.43 (1H, d, J=14.3 Hz, 6α-H), 4.08 (1H, br s, 8-OH). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 234 (M^+, 45), 105 (100).

Gajutsulactone A (**4**): Colorless oil, $[\alpha]_D^{28}$ −128.4° (c=0.10, $CHCl_3$), $[\alpha]_D^{24}$ −181.3° (c=0.1, MeOH). High-resolution EI-MS: Calcd for $C_{15}H_{22}O_2$ (M^+): 234.1620. Found: 234.1606. CD [MeOH, $\Delta\varepsilon$ (nm)]: −11.82 (234). UV [MeOH, nm, (log ε)]: 233 (4.03). IR (film): 2977, 1700, 1646, 1615, 1061 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, s, 15-H_3), 1.69, 1.93 (1H each, both m, 2-H_2), 1.72 (3H, d, J=0.6 Hz, 14-H_3), [1.84 (3H, s), 2.20 (3H, dd, J=1.6, 1.8 Hz), 12, 13-H_3], 1.85, 1.93 (1H each, both m, 3-H_2), 2.06 (1H, m, 5-H), 2.12 (1H, d-like, 6α-H), 2.25 (1H, ddd, J=8.2, 8.2, 8.2 Hz, 1-H), 2.47 (1H, d-like, 6β-H), 4.74, 4.76 (1H each, both d-like, 9-H_2). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 234 (M^+, 10), 107 (100).

Gajutsulactone B (**5**): Colorless oil, $[\alpha]_D^{27}$ −35.0° (c=0.10, $CHCl_3$), $[\alpha]_D^{26}$ −53.4° (c=0.1, MeOH). High-resolution EI-MS: Calcd for $C_{15}H_{22}O_2$ (M^+): 234.1620. Found: 234.1623. CD [MeOH, $\Delta\varepsilon$ (nm)]: −3.03 (239). UV [MeOH, nm, (log ε)]: 232 (4.37). IR (film): 2973, 1713, 1646, 1620, 1067 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.22, 1.78 (3H each, both s, 15, 14-H_3), 1.87, 2.04 (1H each, both m, 2-H_2), 1.94 (2H, m, 3-H_2), [1.85 (3H, s), 2.15 (3H, dd, J=1.5, 1.8 Hz) 12, 13-H_3], 2.24 (1H, d like, 6α-H), 2.30 (1H, m, 5-H), 2.50 (1H, d like, 6β-H), 2.88 (1H, ddd, J=6.4, 6.4, 9.8 Hz, 1-H), 4.84, 5.01 (1H each, both br s, 9-H_2). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 234 (M^+, 5), 107 (100).

Zedoarolide A (**6**): Colorless oil, $[\alpha]_D^{18}$ −32.5° (c=0.10, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{15}H_{21}O_6$ (M+H)$^+$: 297.1338. Found: 297.1340. CD [MeOH, $\Delta\varepsilon$ (nm)]: +5.15 (222), −3.33 (245). UV [MeOH, nm, (log ε)]: 217 (3.81). IR (KBr): 3453, 2962, 1736, 1701, 1686, 1062 cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 1.52, 1.63, 1.80 (3H each, both s, 15, 14, 13-H_3), 1.77 (1H, m, 2α-H), 2.00 (2H, m, 3-H_2), 2.33 (1H, ddd, J=3.9, 11.3, 14.9 Hz, 2β-H), 2.75 (1H, br d, J=*ca.* 12 Hz 5-H), 2.86 (1H, dd, J=11.9, 12.5 Hz, 6α-H), 3.01 (1H, br d, J=*ca.* 13 Hz, 6β-H), 4.84 (1H, s, 9-H). ^{1}H-NMR (CD_3OD) δ: 1.21, 1.43, 1.80 (3H each, both s, 15, 14, 13-H_3), 1.56 (1H, m, 2α-H), 1.71 (1H, ddd, J=1.3, 10.3, 15.3 Hz, 3β-H), 1.82 (1H, m, 3α-H), 2.07 (1H, dd, J=2.1, 12.5 Hz 5-H), 2.21 (1H, dd, J=12.5, 12.5 Hz,

6α-H), 2.22 (1H, br dd, *J*=*ca.* 11, 11 Hz, 2β-H), 2.60 (1H, dd, *J*=2.1, 12.5 Hz, 6β-H), 4.12 (1H, s, 9-H). ^{13}C-NMR (C_5D_5N, CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS *m*/*z*: 297 $(M+H)^+$. Negative-ion FAB-MS *m*/*z*: 295 $(M-H)^-$.

Zedoarolide B (**7**): Colorless oil, $[\alpha]_D^{21}$ −20.6° (*c*=1.80, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{15}H_{23}O_5$ $(M+H)^+$: 283.1546. Found: 283.1530. CD [MeOH, $\Delta\varepsilon$ (nm)]: +1.76 (226), −3.64 (247). UV [MeOH, nm, (log ε)]: 223 (3.82). IR (KBr): 3475, 2940, 1719, 1686, 1000 cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 1.43, 1.57, 1.80 (3H each, both s, 14, 15, 13-H_3), 1.79 (1H, m, 2α-H), 1.97 (1H, m, 3α-H), 1.98 (1H, m, 2β-H), 2.08 (1H, m, 3β-H), 2.43 (1H, dd, *J*=12.8, 12.8 Hz 6β-H), 2.61 (1H, ddd, *J*=3.7, 3.7, 12.8 Hz, 5-H), 2.80, 2.86 (2H, ABq, *J*=15.5 Hz, 9β, 9α-H), 2.82 (1H, dd, *J*=3.7, 12.8 Hz, 6α-H), 3.35 (1H, ddd, *J*=3.7, 7.6, 7.6 Hz, 1-H). ^{1}H-NMR (CD_3OD) δ: 1.20, 1.33, 1.79 (3H each, both s, 14, 15, 13-H_3), 1.58 (1H, m, 2α-H), 1.72 (1H, m, 3β-H), 1.82 (1H, m, 2β-H), 1.83 (1H, m, 3α-H), 2.00 (1H, dd, *J*=7.0, 14.9 Hz, 6β-H), 2.01 (1H, br dd, *J*=*ca.* 2, 7 Hz, 5-H), 2.22, 2.33 (2H, ABq, *J*=15.3 Hz, 9β, 9α-H), 2.61 (1H, dd, *J*=2.3, 14.9 Hz, 6α-H), 2.66 (1H, m, 1-H). ^{13}C-NMR (C_5D_5N, CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS *m*/*z*: 283 $(M+H)^+$. Negative-ion FAB-MS *m*/*z*: 281 $(M-H)^-$.

Hydrogenation of 3 and 27 A solution of **3** (4 mg, 0.017 mmol) in MeOH (1.5 ml) was hydrogenated with H_2 in the presence of 10% Pd–C (4 mg) at room temperature for 5 h. The catalyst was filtrated off, and the solvent of the filtrate was evaporated under reduced pressure to give a product mixture. The product mixture was purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O (85 : 15, v/v)] to furnish **3a** (1.5 mg, 37%), and **3b** (1.0 mg, 25%). Through a similar procedure, **27** (82 mg, 0.35 mmol) in MeOH (5 mg) was treated with 10% Pd–C (20 mg), and the mixture was stirred at room temperature for 2 h and furnished **3a** (20 mg, 24%) and **3b** (30 mg, 36%).

3a: Colorless oil, $[\alpha]_D^{29}$ +23.1° (*c*=0.10, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{24}O_2$ (M^+): 236.1776. Found: 236.1773. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, d, *J*=7.0 Hz, 14-H_3), 0.97 (3H, d, *J*=6.7 Hz, 15-H_3), 1.45 (1H, m, 2β-H), 1.47 (1H. m, 3α-H), 1.51 (1H, m, 2α-H), 1.53 (1H, m, 1-H), 1.56 (H, m, 9β-H), [1.66 (3H, s), 1.82 (3H, dd, *J*=1.5, 2.4 Hz), 12, 13-H_3)], 1.78 (1H, dd, *J*=5.5, 12.2 Hz, 9α-H), 1.85 (1H, m, 3β-H), 1.92 (1H, m, 4-H), 1.98 (1H, m, 10-H), 2.03 (1H, ddd, *J*=1.5, 2.4, 13.7 Hz, 6α-H), 2.57 (1H, ddd, *J*=2.4, 4.5, 13.7 Hz, 6β-H). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. EI-MS *m*/*z* (%): 236 (M^+, 17), 193 (100).

3b: Colorless oil, $[\alpha]_D^{29}$ +39.3° (*c*=0.10, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{26}O_2$ (M^+): 238.1933. Found: 238.1925. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, d, *J*=6.8 Hz, 14-H_3), 0.91, 0.93 (3H each, both d, *J*=6.4 Hz, 12, 13-H_3), 0.96 (3H, d, *J*=6.1 Hz, 15-H_3), 1.35 (H, dd, *J*=12.2, 12.5 Hz, 9β-H), 1.42 (1H, m, 2β-H), 1.46 (1H, dd, *J*=9.2, 12.8 Hz, 6α-H), 1.50 (1H. m, 3α-H), 1.53 (1H, m, 2α-H), 1.55 (1H, m, 1-H), 1.62 (1H, dd, *J*=5.8, 12.5 Hz, 9α-H), 1.75 (1H, dd, *J*=6.1, 12.8 Hz, 6β-H), 1.88 (1H, m, 3β-H), 1.90 (1H, m, 4-H), 1.97 (1H, dq, *J*=6.7, 6.4 Hz, 11-H), 2.02 (1H, ddd, *J*=6.1, 6.7, 9.2 Hz, 7-H), 2.11 (1H, dddq, *J*=5.8, 6.7, 12.2, 6.8 Hz, 10-H). ^{13}C-NMR ($CDCl_3$) δ_C: given in Table 1. EI-MS *m*/*z* (%): 238 (M^+, 3), 95 (100).

Acetylation of Zedoarolide A (6) A solution of **6** (1.0 mg, 3.4 μmol) in pyridine (1.0 ml) was treated with Ac_2O (0.8 ml) in the presence of 4-DMAP and the mixture was stirred at room temperature for 30 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtrated. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O (60 : 40, v/v)] to furnish **6a** (1.4 mg, 98%).

6a: High-resolution positive-ion FAB-MS: Calcd for $C_{21}H_{27}O_9$ $(M+H)^+$: 423.1655. Found: 423.1677. ^{1}H-NMR (CD_3OD) δ: 1.36, 1.50 (3H each, both s, 15, 14-H_3), 1.63 (2H, m, 2α, 3β-H), 1.86 (3H, d, *J*=1.3 Hz, 13-H_3), 1.98, 2.04, 2.11 (3H each, both s, –OAc), 2.19 (1H, ddd, *J*=1.3, 2.6, 13.0 Hz 5-H), 2.28 (1H, ddd, *J*=2.5, 10.3, 12.8 Hz, 3α-H), 2.43 (1H, dd, *J*=13.0, 13.0 Hz, 6α-H), 2.50 (1H, ddd, *J*=2.5, 8.9, 11.5 Hz, 2β-H), 2.79 (1H, dd, *J*=2.6, 13.0 Hz, 6β-H), 4.84 (1H, br s, 9-H). ^{13}C-NMR (CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS *m*/*z*: 423 $(M+H)^+$. Negative-ion FAB-MS *m*/*z*: 421 $(M-H)^-$.

Bioassay: NO Production from Macrophages Stimulated by LPS Peritoneal exudate cells were collected from the peritoneal cavities of male ddY mice by washing with 6—7 ml of ice-cold phosphate-buffered saline (PBS), and cells (5×10^5 cells/well) were suspended in 200 μl of RPMI 1640 supplemented with 5% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml), and pre-cultured 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 (more than 95% macrophages as determined by Giemsa staining) were cultured in fresh medium containing 10 μg/ml LPS and test compound (10, 30, 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 by 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 0.04 M HCl was then added to dissolve the formazan produced by the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm (reference, 655 nm). L-NMMA was used as a reference compound. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated by 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 (−)].

References and Notes

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