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INHIBITORS OF NITRIC OXIDE PRODUCTION AND NEW SESQUITERPENES, 4-*EPI*-CURCUMENOL, NEOCURCUMENOL, GAJUTSULACTONES A AND B, AND ZEDOAROLIDES A AND B FROM ZEDOARIAE RHIZOMA

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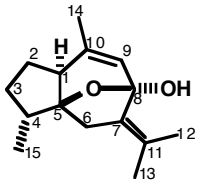
Abstract — Six new guaiane- or secoguaiane-type sesquiterpenes, 4-*epi*-curcumenol, neocurcumenol, gajutsulactones A and B, and zedoarolides A and B, were isolated from aqueous acetone extract of Zedoariae Rhizoma (Zingiberaceae), together with 34 sesquiterpenes and two diarylheptanoids. The stereostructures of new sesquiterpenes were elucidated on the basis of chemical and physicochemical evidence, which included nuclear Overhauser effect (NOE) and circular dichroic (CD) spectroscopic analyses. Gajutsulactones A and B, and 14 sesquiterpenes, and two diarylheptanoids were found to inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.

In the course of our studies on the bioactive constituents of Zedoariae Rhizoma,[1] we have isolated two guaiane-type sesquiterpenes, 4-*epi*-curcumenol (**1**), and neocurcumenol (**2**), and two secoguaiane-type sesquiterpenes, gajutsulactones A (**3**) and B (**4**), from the ethyl acetate-soluble portion. Furthermore, from the 1-butanol-soluble portion, we isolated two new polyoxygenated guaiane-type sesquiterpenes termed zedoarolides A (**5**) and B (**6**). This paper deals with the structure elucidation of new sesquiterpenes (**1**—**6**) from Zedoariae Rhizoma and the inhibitory effect of the constituents from this herbal medicine on NO production.

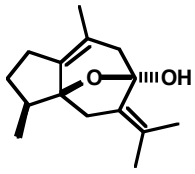
The 80% aqueous acetone extract of Zedoariae Rhizoma (cultivated in Szechwan province, China) was partitioned into a mixture of ethyl acetate and water to furnish the ethyl acetate-soluble portion and aqueous phase. The aqueous phase was further extracted with 1-butanol to give the 1-butanol-soluble portion and water-soluble portion. The ethyl acetate-soluble portion was subjected to silica gel and octadecyl silica gel (ODS) column chromatography and finally HPLC to furnish 4-epicurcumenol (**1**, 0.0006% from natural medicine), neocurcumenol (**2**, 0.0014%), and gajutsulactones A (**3**, 0.0002%) and B (**4**, 0.0002%) together with thirty sesquiterpenes (**7**—**30**, **34**, **35**, **37**—**40**) and a diarylheptanoid, curcumin (**41**). The 1-butanol-soluble portion was also separated by above chromatography to give zedoarolides A (**5**, 0.0002%) and B (**6**, 0.0007%) together with five sesquiterpenes (**30**—**33**, **36**) and a diarylheptanoid, bis(4-hydroxycinnamoyl)methane (**42**).

4-Epicurcumenol (**1**), colorless oil, $[\alpha]_D^{26}$ +120.1° (*c*=1.8, $CHCl_3$), $C_{15}H_{22}O_2$,[2] showed absorption bands at 3370, 1663, and 1065 cm^{-1} due to hydroxyl, olefin, and ether functions in the IR spectrum. In the EI-MS of **1**, molecular ion peak was observed at *m*/*z* 234 (M^+, 30), while the ^{1}H-[3] and ^{13}C-NMR ($CDCl_3$, Table 1) of **1** showed signals assignable to a secondary methyl [δ 0.95 (d, *J*=6.9 Hz, 15-H_3)], three methyls [δ 1.59, 1.80 (both s, 12 and 13-H_3), 1.67 (s, 14-H_3)], and an olefin [δ 5.78 (br s, 9-H)] together with three methylenes (2, 3, 6-H_2), two methines (1, 4-H), and five quaternary carbons (5, 7, 8, 10, 11-C). The connectivities of ^{1}H–^{1}H and the quaternary carbons in **1** were clarified by various 2D-NMR experiments[4] as shown in Figure 1. The proton and carbon signals in the NMR spectra of **1** were superimposable on those of curcumenol (**24**),[5] which was a principle guaiane-type sesquiterpene from Zedoariae Rhizoma, except for the signals due to the 4-position.

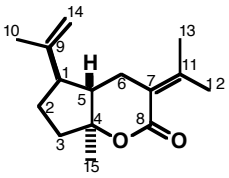
and 3β-H; 3α-H and 15-H_3; 4-H and 6β-H; 15-H_3 and 6α-H, 6β-H) as shown in Figure 1. On the basis of this evidence, the stereostructure of **1** was elucidated to be the 4-position isomer of **24**.



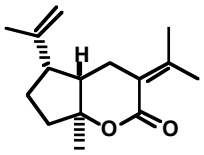
4-epicurcumenol (**1**)



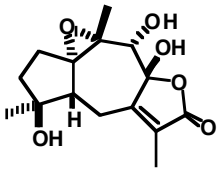
neocurcumenol (**2**)



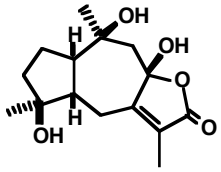
gajutsulactone A (**3**)



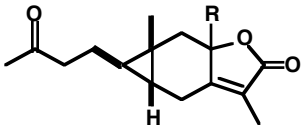
gajutsulactone B (**4**)



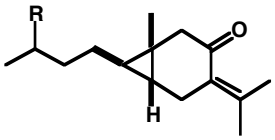
zedoarolide A (**5**)



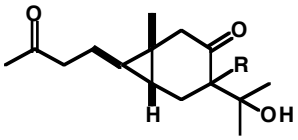
zedoarolide B (**6**)



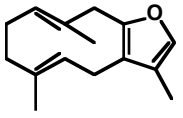
curcumenolactone A (**7**) : R=β-H
curcumenolactone B (**8**) : R=α-H
curcumenolactone C (**9**) : R=α-OH



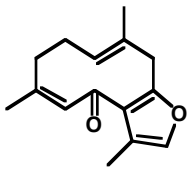
curcumenone (**10**) : R= =O
4*S*-dihydrocurcumenone (**11**) : R=α-OH



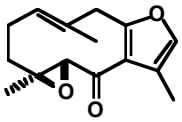
curcarabranol A (**12**) : R=β-H
curcarabranol B (**13**) : R=α-H



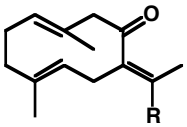
furanodiene (**14**)



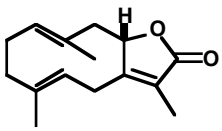
isofuranodienone (**15**)



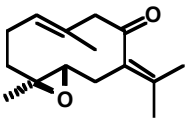
zederone (**16**)



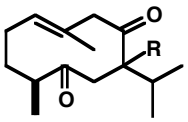
germacrone (**17**) : R=H
13-hydroxygermacrone (**18**) : R=CH_2OH



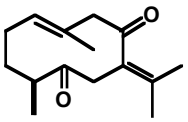
glechomanolide (**19**)



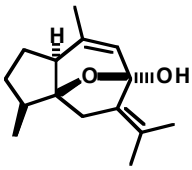
(+)-germacrone 4,5-epoxide (**20**)



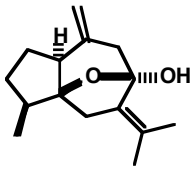
curdione (**21**) : R=β-H
neocurdione (**22**) : R=α-H



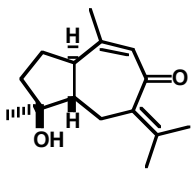
dehydrocurdione (**23**)



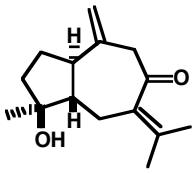
curcumenol (**24**)



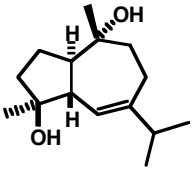
isocurcumenol (**25**)



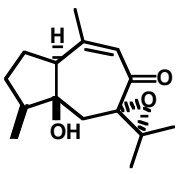
procurcumenol (**26**)



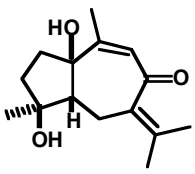
isoprocurcumenol (**27**)



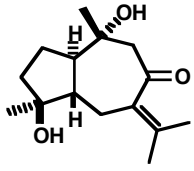
alismoxide (**28**)



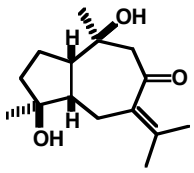
7α,11α-epoxy-5β-hydroxy-9-guaiaen-8-one (**29**)



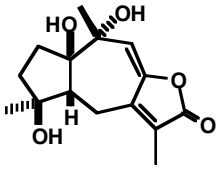
aerugidiol (**30**)



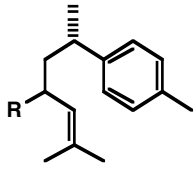
zedoarondiol (**31**)



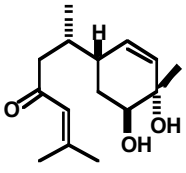
isozedoarondiol (**32**)



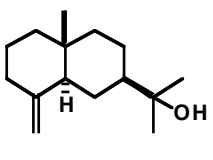
zedoalactone B (**33**)



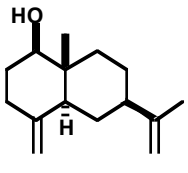
(+)-*ar*-turmerone (**34**) : R= =O
bisacumol (**35**) : R=α-OH



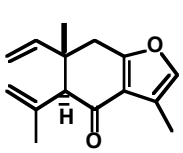
bisacurone (**36**)



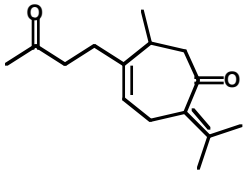
β-eudesmol (**37**)



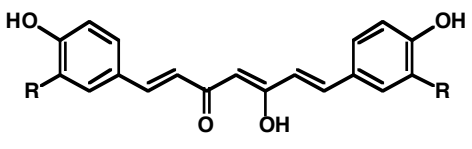
β-dictyopterol (**38**)



curzerenone (**39**)



curcumadione (**40**)



curcumin (**41**) : R=OMe
bis(4-hydroxycinnamoyl)methane (**42**) : R=H

Chart 1

Neocurcumenol (**2**), colorless oil, $[\alpha]_D^{25}$ +15.3° (*c*=2.0, $CHCl_3$), $C_{15}H_{22}O_2$,[2] EI-MS *m/z*: 234 (M^+, 45), 105 (base peak), showed absorption bands at 3379, 1694, and 1051 cm^{-1} due to hydroxyl, olefin, and ether functions in the IR spectrum. In the ^{1}H-[6] and ^{13}C-NMR ($CDCl_3$, Table 1) spectra[4] of **2** were also found to be similar to those of **1** and **24**, except for lacking an olefin proton. As shown in Figure 1, the plane structure of **2** was elucidated on the basis of HMBC experiment and the relative stereostrucutre of **2** was characterized by NOESY experiment, which showed NOE correlations between the following proton pairs (2α-H and 3α-H; 2β-H and 3β-H; 4-H and 6α-H, 6β-H; 15-H_3 and 3β-H, 6β-H) as shown in Figure 1. Catalytic hydrogenation of **2** furnished **2a**[7] and **2b**,[7] whose stereostructures were determined by NOESY experiment. On the other hand, **2a** and **2b** were also obtained by hydrogenatioin of **24** by the same way. On the basis of this evidence, the stereostructure of **2** was elucidated to be the $\Delta^{1(10)}$-isomer of **24** (Figure 2).

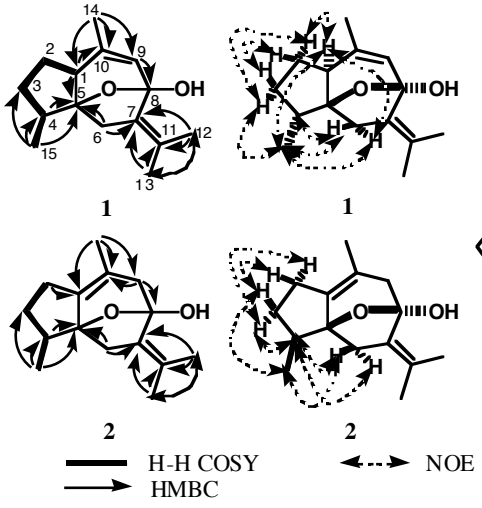


Figure 1

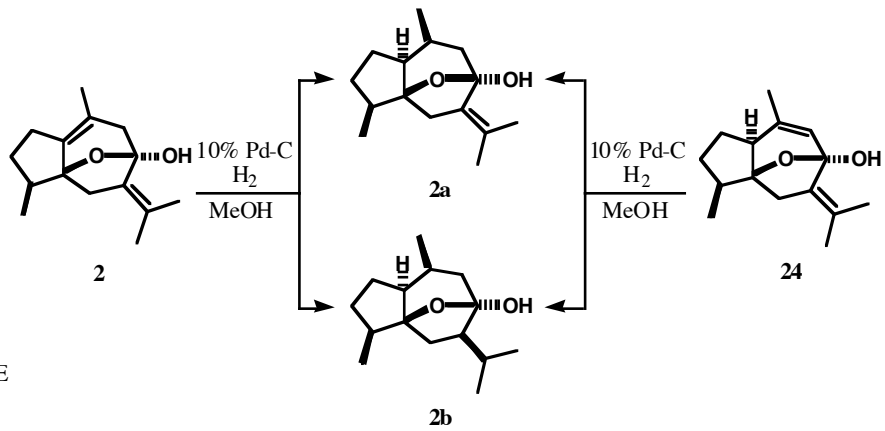


Figure 2

The UV spectrum of gajutsulactone A (**3**), colorless oil, $[\alpha]_D^{28}$ –128.4° (*c*=0.1, $CHCl_3$), $C_{15}H_{22}O_2$,[2] CD $\Delta\varepsilon$ (MeOH, nm): –11.82 (234), showed an absorption maximum at 233 nm (log ε 4.03 in MeOH), suggestive of an α,β-unsaturated lactone moiety, while the IR spectrum showed absorption bands due to α,β-unsaturated ester carbonyl, olefin, and ether groups at 1700, 1646, 1615, and 1061 cm^{-1}. The ^{1}H-[8] and ^{13}C-NMR ($CDCl_3$, Table 1) spectra[4] of **3** indicated the presence of four methyl {δ 1.23 (s, 15-H_3), 1.72 (d, *J*=0.6 Hz, 14-H_3), and [1.84 (s), 2.22 (dd, *J*=1.6, 1.8 Hz), 12 and 13-H_3]}, and an *exo*-methylene [δ 4.74, 4.76 (1H each, both d like, 9-H_2)] together with three methylenes (2, 3, 6-H_2), two methines (1, 5-H), and five quaternary carbons (4, 7, 8, 10, 11-C). Various 2D-NMR data as shown in Figure 3 led us to confirm the skeleton of **3** to be 8,9-seco-7(11),9(10)-guaiadien-8,4-olide. The stereostructure of **3** was elucidated by NOESY experiment, in which the NOE correlations of **3** were observed between the following proton pairs (1-H and 6α-H, 15-H_3; 5-H and 6β-H). Next, the stereostructure of gatjutsulactone B (**4**),[9] which was the 1-position isomer of **3**, was also characterized by NOSEY experiment (NOE: 1-H and 5-H, 6β-H).

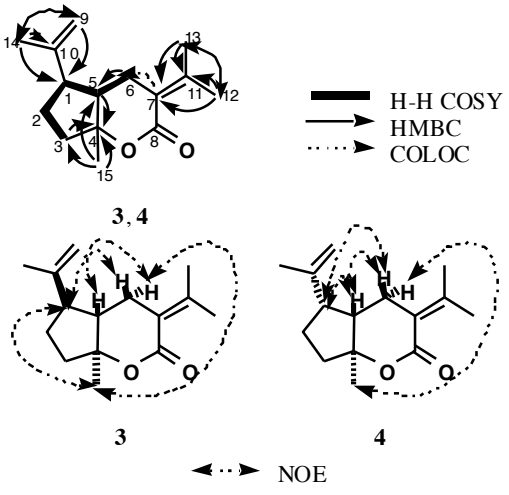


Figure 3

Zedoarolide A (**5**), colorless oil, $[\alpha]_D^{18}$ –32.5° (*c*=0.1, MeOH), $C_{15}H_{20}O_6$,[2] FAB-MS *m/z*: 297 $(M+H)^+$, 295 $(M-H)^-$ showed absorption bands at 3453, 1736, 1686, and 1062 cm^{-1} ascribable to hydroxyl, α,β-unsaturated γ-lactone, olefin, and ether functions in the IR spectrum, while its UV spectrum also indicated the presence of an α,β-unsaturated γ-lactone chromophore

from the absorption maximum at 217 nm (log ε 3.81 in MeOH). The ^{1}H-[10] and ^{13}C-NMR (C_5D_5N, Table 1) spectra[4] of **5** showed signals assignable to three methyls [δ 1.52, 1.63, 1.80 (both s, 14, 15, and 13-H_3)], and a methine bearing a oxygen function [δ 4.84 (s, 9-H)] together with three methylenes (2, 3, 6-H_2), a methine (5-H), and seven quaternary carbons (1, 4, 7, 8, 10, 11, 12-C). In order to clarify the number of hydroxyl groups, acetylation of **5** furnished triacetate derivative (**5a**).[7] On the basis of the NMR data of **5** and **5a**, the plane structure of **5** was characterized to be 1,10-epoxy-4,8,9-trihydroxy-7(11)-guaien-12,8-olide (Figure 4).

Table 1. ^{13}C-NMR Data for **1**—**6**

	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[b]
C-1	48.8	137.6	47.5	42.6	70.3	53.2
C-2	28.3	23.4	26.7	26.3	29.6	25.3
C-3	31.6	29.8	36.7	38.2	39.5	38.3
C-4	41.5	39.4	85.2	85.3	78.7	80.8
C-5	87.7	86.4	45.7	45.9	50.8	52.4
C-6	37.5	44.3	26.9	25.8	20.9	24.6
C-7	122.2	126.5	119.7	120.5	159.0	161.5
C-8	101.3	104.1	167.2	167.4	109.3	106.9
C-9	125.9	42.4	110.5	111.9	77.6	44.1
C-10	138.4	123.5	145.8	145.3	62.3	72.2
C-11	137.1	136.2	152.1	151.6	125.7	122.8
C-12	*19.0	*19.4	*23.3	*23.3	173.0	173.7
C-13	*22.2	*22.6	*23.6	*23.5	8.3	8.0
C-14	21.1	18.3	19.5	25.1	22.1	32.5
C-15	16.8	15.0	19.5	20.0	22.5	25.6

Measured in [a] $CDCl_3$ and [b] C_5D_5N at 125 MHz.
* May be interchangeable within the same column.

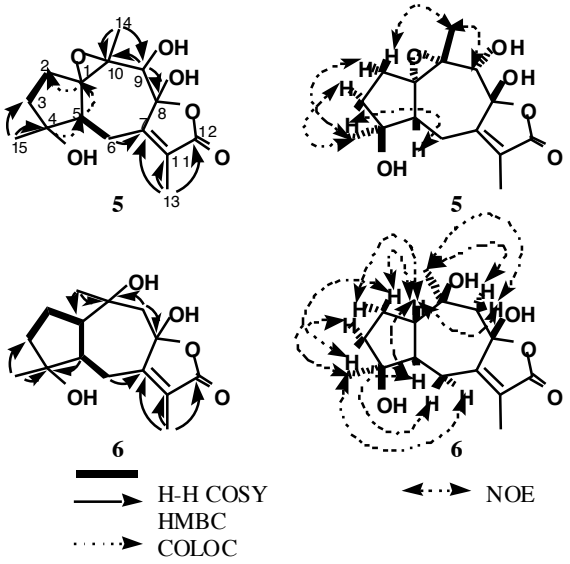


Figure 4

The NOESY experiment on **5** showed NOE correlations between the following proton pairs (2α-H, 3α-H and 15-H_3; 3β-H and 5-H; 14-H_3 and 2β-H, 9-H), as depicted in Figure 4. On the basis of these findings, the stereostructure of **5** was elucidated.

Zedoarolide B (**6**), colorless oil, $[\alpha]_D^{21}$ –20.6° (c=1.8, MeOH), $C_{15}H_{22}O_5$,[2] FAB-MS m/z: 283 $(M+H)^+$, 281 $(M-H)^-$, UV [MeOH, (log ε)]: 223 nm (3.82), showed absorption bands at 3475, 1719, 1686, and 1000 cm^{-1} due to hydroxyl, α,β-unsaturated γ-lactone, olefin, and ether functions in the IR spectrum. Various NMR experiments[4] in the ^{1}H-[11] and ^{13}C-NMR (C_5D_5N, Table 1) spectra of **6** led us to clarify the plane structure to be 4,8,10-trihydroxy-7(11)-guaien-12,8-olide, and the stereostructure of **6** was also characterized by NOESY experiment, as shown in Figure 4.

Finally, the absolute stereostructures of **5** and **6** were determined by CD spectrum on α,β-unsatulated γ-lactone moiety.[1c,12] Namely, the CD spectrum of **5** showed the characteristic Cotton curve [$\Delta\varepsilon$ +5.15 (222 nm), –3.33 (245 nm) in MeOH] for the 8*R* configuration in *endo*-α,β-unsatulated γ-lactones. On the other hand, the CD data of **6** showed the Cotton curve [$\Delta\varepsilon$ +1.76 (226 nm), –3.64 (247 nm) in MeOH], which were similar to those of **5**, so that the absolute stereostructure of **6** was determined to be 8*S* configuration.

The inhibitory effects of constituents from Zedoariae Rhizoma against NO production were examined using lipopolysaccharide (LPS)-activated mouse peritoneal macrophages.[13] Sixteen sesquiterpenes, such as gajutsulactones A (**3**) and B (**4**), curcumenone (**10**), furanodiene (**14**), isofuranodienone (**15**), 13-hydroxygermacrone (**18**), glechomanolide (**19**), neocurdione (**22**), curcumenol (**24**), isocurcumenol (**25**), procurcumenol (**26**), (+)-*ar*-turmerone (**34**), bisacumol (**35**), bisacurone (**36**), β-eudesmol (**37**), and β-dictyopterol (**38**) were found to inhibit overproduction of NO [50.4 ~ 98.5% inhibition at 100 μM] and two diarylheptanoids, curcumin (**41**, 83.8% at 30 μM), and bis(4-hydroxycinnamoyl)methane (**42**, 57.1% at 100 μM) as shown in Table 2. The inhibitory activity of these compounds 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.

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

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2 The molecular composition of the compound given with the chemical formula was determined by high-resolution EI-MS or FAB-MS.

3 **1**: High-resolution EI-MS: Calcd for $C_{15}H_{22}O_2$ (M^+) : 234.1620. Found : 234.1620. ^{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 and 13-H_3), 1.67 (3H, s, 14-H_3), 1.94 (2H, m, 1 and 2α-H), 2.15 (2H, m, 3β and 4-H), 2.18, 2.66 (1H each, both d, *J*=15.2 Hz, 6β and 6α-H), 3.38 (1H, br s, 8-OH), 5.78 (1H, br s, 9-H).

4 The ^{1}H- and ^{13}C-NMR spectra of new compounds (**1**—**6**) and their derivatives (**2a**, **2b**, and **5a**) were assigned with the aid of homo-and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuculear multiple bond connectivity (HMBC) experiments. Furthermore, **3**—**6** were also assigned with aid of correlation *via* long-range coupling (COLOC) experiment.

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6 **2**: High-resolution EI-MS: Calcd for $C_{15}H_{22}O_2$ (M^+) : 234.1620. Found : 234.1623. ^{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 and 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).

7 All new compounds (**2a**, **2b**, **5a**) were chracterized by physicochemical properties and full characteristics will be presented in a full paper.

8 **3**: High-resolution EI-MS: Calcd for $C_{15}H_{22}O_2$ (M^+) : 234.1620. Found : 234.1606. ^{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 and 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).

9 **4**: Colorless oil, $[\alpha]_D^{27}$ –35.0° (*c*=0.1, $CHCl_3$). 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): 1713, 1646, 1620, 1067 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 1.22, 1.78 (3H each, both s, 15 and 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 and 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). EI-MS *m*/*z* (%): 234 (M^+, 5), 107 (100).

10 **5**: High-resolution FAB-MS: Calcd for $C_{15}H_{21}O_6$ $(M+H)^+$: 297.1338. Found : 297.1340. ^{1}H-NMR (C_5D_5N) δ : 1.52, 1.63, 1.80 (3H each, both s, 15, 14, and 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).

11 **6**: High-resolution FAB-MS: Calcd for $C_{15}H_{23}O_5$ $(M+H)^+$: 283.1546. Found : 283.1530. ^{1}H-NMR (C_5D_5N) δ : 1.43, 1.57, 1.80 (3H each, both s, 14, 15, and 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 and 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).

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