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Absolute stereostructure of carabrane-type sesquiterpene and vasorelaxant-active sesquiterpenes from Zedoariae Rhizoma

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Abstract—The aqueous acetone extract of Zedoariae Rhizoma was found to show inhibitory effects on contractions induced by high concentrations of potassium cation (K^+) in isolated rat aortic strips. From the extract, two new carabrane-type sesquiterpenes, curcarabranols A and B, were isolated together with a few carabrane-type sesquiterpenes, such as curcumenone and 4*S*-dihydrocurcumenone. Their absolute stereostructures were determined on the basis of chemical and physicochemical evidence, which included the application of the modified Mosher's method and chemical conversion from curcumenone to curcarabranols A and B. In addition, several sesquiterpenes and diarylheptanoids (e.g. germacrone, glechomanolide, isocurucmenol, β-eudesmol, and β-dictyopterol) showed potent inhibitory effects on contractions induced by high concentrations of K^+ in isolated rat aortic strips (inhibition >80% at 100 μM), while they did not inhibit norepinephrine-induced contractions, so that the vasorelaxant activities of these sesquiterpenes were presumed to be dependent on their calcium channel-blocking activity.

1. Introduction

The Zingiberaceae plant *Curcuma zedoaria* ROSCOE (the common name, Zedoary) has been widely cultivated as a vegetable or spice in South and Southeast Asian countries. The rhizomes of this plant (Zedoariae Rhizoma) are used as a stimulant, stomachic, carminative, diuretic, anti-diarrheal, anti-emetic, anti-pyretic, depurator, and also to clean and cure ulcers, wounds, and other kinds of skin disorders in India and Southeast Asian countries. In Japanese and Chinese traditional medicines, Zedoariae Rhizoma is prescribed as a stomachic, emmenagogue, and for the treatment of 'Oketsu' syndrome caused by blood stagnation in various traditional preparations. During the course of our characterization studies on the bioactive constituents from Zingiberaceae plants,[1] we found that the 80% acetone extract of Zedoariae Rhizoma showed vasorelaxant,[2] hepatoprotective,[3] and nitric oxide (NO) production inhibitory activities.[4] From the aqueous acetone extract, we have isolated eleven sesquiterpenes called curcarabranols A (**3**) and B (**4**), curcumenolactones A (**5**), B (**6**), and C (**7**), 4-epicurcumenol (**19**), neocurcumenol (**21**), gajutsulactones A (**30**) and B (**31**), and zedoarolides A (**32**) and B (**33**). In the preceding papers,[3,4] we reported the hepatoprotective and NO production inhibitory activities of the sesquiterpene and diarylheptanoid constituents and the structure elucidation of **5**–**7**, **19**, **21**, and **30**–**33**. This paper presents a full account of the absolute stereostructure elucidation of carabrane-type sesquiterpenes, curcumenone (**1**), 4*S*-dihydrocurcumenone (**2**), and curcarabranols A (**3**) and B (**4**), which included the conversion of curcumenone (**1**) into curcarbranols A (**3**) and B (**4**). In addition, we describe vasorelaxant effects of the constituents on contractions induced by high concentrations of potassium cation (high K^+) and DL-norepinephrine (NE) in isolated rat aortic strips.[2]

2. Results and discussion

The isolation of the chemical constituents from Zedoariae Rhizoma was carried out through the following procedure. Zedoariae Rhizoma (cultivated in Szechwan province, China, purchased from Tochimoto Tenkaido Co., Ltd, Osaka) was extracted with 80% aqueous acetone at room temperature. The relaxant effects of the 80% aqueous acetone extract on high K^+- and NE-induced contractions in rat thoracic aorta were examined. Cumulative application of the 80% aqueous acetone extract (6.25–50 μg/ml) inhibited the sustained contractions induced by high K^+. The IC_{50} value of the 80% aqueous acetone extract was 18 μg/ml as shown in Table 1, and that of nifedipine as a reference drug was 6.4 nM. On the other hand, the 80% aqueous acetone extract did not inhibit the contractions induced by NE in isolated rat aortic strips.

The 80% aqueous acetone extract was partitioned in an ethyl acetate and water mixture to give an ethyl acetate-soluble portion and an aqueous phase. The aqueous phase was

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Table 1. Inhibitory effects of 80% aqueous acetone extracts of Zedoariae Rhizoma on the contractions induced by high K^+ concentration and norepinephrine in isolated rat thoracic aorta

	Inhibition (%)			
	High K^+ (54 mM)			
	6.25 μg/ml	12.5 μg/ml	25.0 μg/ml	50.0 μg/ml
80% Aqueous acetone extract	15.8±3.4**	32.2±2.7**	69.8±6.2**	98.8±6.5**
	3 nM	10 nM	30 nM	100 nM
Nifedipine	28.4±4.2**	64.1±4.4**	80.6±3.4**	84.0±3.7**

		Contraction (%)		
		DL-Norepinephrine		
	Conc.	10 nM	100 nM	1 μM
Control		21.6±2.3	67.0±2.7	100.0±8.8
80% Aqueous acetone extract	50.0 μg/ml	31.9±2.2	75.1±5.3	105.3±5.2
Prazosin	10^{-10} M	9.9±1.4**	46.6±5.6*	72.2±9.7
	10^{-9} M	0.8±0.7**	9.9±6.4**	41.0±9.5**

Each value represents the mean±SEM (n=4–5). Significantly different from the control: *$p<0.05$, **$p<0.01$.

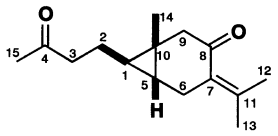
further extracted with 1-butanol to give a 1-butanol-soluble portion and a water-soluble portion. The ethyl acetate-soluble portion was subjected to silica gel, silver nitrate-treated silica gel, and ODS column chromatography and finally HPLC to furnish curcumenone (**1**,[5] 0.041% from the natural medicine), 4*S*-dihydrocurcumenone (**2**,[6] 0.0011%), and curcarabranols A (**3**,[2] 0.00030%) and B (**4**,[2] 0.00030%), together with 30 sesquiterpenes (**5**–**26**, **30**, **31**, **34**, **35**, **37**–**40**) and diarylheptanoid, curcumin (**41**). The 1-butanol-soluble portion was also separated by the above-mentioned chromatography to give seven sesquiterpenes (**26**–**29**, **32**, **33**, **36**) and bis(4-hydroxycinnamoyl)methane (**42**) (see Scheme 1). The details of the isolation procedure were described previously.[3]

2.1. Absolute stereostructures of curcumenone (1) and 4*S*-dihydrocurcumenone (2)

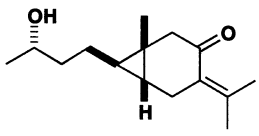
Curcumenone (**1**) has been previously isolated as a principal carabrane-type sesquiterpene from Zedoariae Rhizoma and its relative stereostructure was clarified by nuclear Overhauser effect spectroscopy (NOESY) experiments.[5] The absolute stereostructure of **1** was deduced on the basis of biotransformation[6–8] and hypothetical biogenesis[5] from germacrone (**11**) to **1** via (4*S*,5*S*)-germacrone 4,5-epoxide. However, the absolute configuration of the germacrone 4,5-epoxide was revised to the (4*S*,5*R*) configuration (**14**),[9] so that the absolute stereostructure of **1** was concluded as being left unclarified (Scheme 2). In order to determine the absolute stereostructure of **1**, we carried out the chemical modification from **1** to **1d** and the application of the modified Mosher's method.[10]

Thus, **1** was treated with 1,2-bis(trimethylsilyloxy)ethane and trimethylsilyl trifluoromethane sulfonate (TMSOTf)[11] to give the 4-acetal derivative (**1a**) in 86% yield. The 4-acetal derivative (**1a**) was subjected to $NaBH_4$ reduction in the presence of cerium chloride ($CeCl_3 \cdot 6H_2O$) to yield the 8α-hydroxyl (**1b**, 68% yield) and the 8β-hydroxyl (**1c**, 23% yield) derivatives. Hydrogenation of **1b** furnished **1d** in 45% yield. The stereostructures of the 8-hydroxyl groups in **1b** and **1c** and the 7-isopropyl group in **1d** were clarified by the NOESY experiment, which showed nuclear Overhauser effect (NOE) correlations between the following proton pairs [(**1b**: 1-H and 6α-H; 5-H and 6β-H, 9β-H; 8-H and 9β-H, 14-H_3; 9β-H and 14-H_3), (**1c**: 1-H and 9α-H; 5-H and 6β-H; 8-H and 9α-H; 9β-H and 14-H_3), (**1d**: 1-H and 7-H; 5-H and 8-H, 14-H_3; 8-H and 9β-H, 14-H_3; 9β-H and 14-H_3), as shown in Fig. 1]. Finally, the absolute stereostructure of the 8-hydroxyl group in **1d** was determined by application of the modified Mosher's method[10] for the (*R*)- and (*S*)-2-methoxy-2-trifluorophenylacetate (MTPA esters, **1e** and **1f**), which were prepared by treatment of **1d** with (*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl) and 4-dimethyl-aminopyridine (4-DMAP). As shown in Fig. 1, the signals due to protons on the 6, 7, 11, 12 and 13-carbons in the (*S*)-MTPA ester (**1f**) were observed at a lower field than those of (*R*)-MTPA ester (**1e**) ($\Delta\delta$, positive), while the signals due to protons on the 1, 9 and 14-carbons in **1f** were observed at higher fields than those of **1e** ($\Delta\delta$, negative). Thus, the absolute configuration at the 8-position in **1d** has been shown to be *S*. Consequently, the absolute stereostructure of **1** was determined.

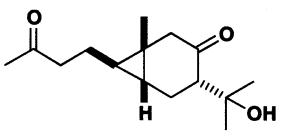
4*S*-Dihydrocurcumenone (**2**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{27}=-5.1°$). The electron impact (EI)-MS of **2** showed a molecular ion (M^+) peak at *m*/*z* 236 in addition to fragment ion peaks at *m*/*z* 218 (M^+-H_2O) and *m*/*z* 68 (base peak) and the high-resolution MS analysis of the molecular ion peak revealed the molecular formula of **2** to be $C_{15}H_{24}O_2$. The IR spectrum of **2** showed absorption bands at 3436, 1678, and 1053 cm^{-1}, which were assigned to hydroxyl, conjugated carbonyl, and cyclopropane functions, respectively. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 2) spectra of **2** showed signals assignable to a cyclopropane [δ 0.46 (dt, J=4.9, 6.4 Hz, 1-H), 0.65 (m, 5-H)], four methyls [δ 1.12 (s, 14-H_3), 1.18 (d, J=6.1 Hz, 15-H_3), 1.79, 2.09 (both s, 12 and 13-H_3)], and a



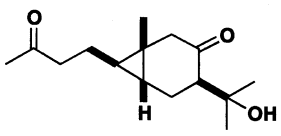
curcumenone (**1**)



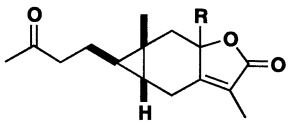
4*S*-dihydrocurcumenone (**2**)



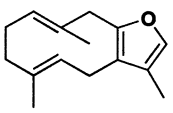
curcarabranol A (**3**)



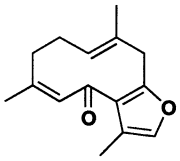
curcarabranol B (**4**)



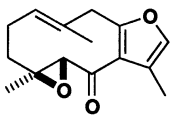
curcumenolactone A (**5**) : R=β-H
curcumenolactone B (**6**) : R=α-H
curcumenolactone C (**7**) : R=α-OH



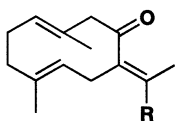
furanodiene (**8**)



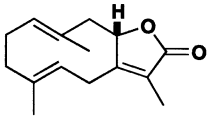
isofuranodienone (**9**)



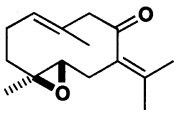
zederone (**10**)



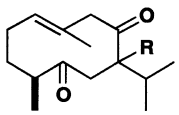
germacrone (**11**) : R=H
13-hydroxygermacrone (**12**) : R=CH_2OH



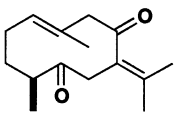
glechomanolide (**13**)



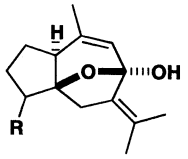
(+)-germacrone 4,5-epoxide (**14**)



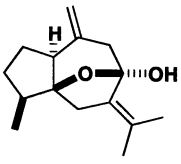
curdione (**15**) : R=β-H
neocurdione (**16**) : R=α-H



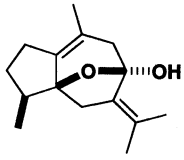
dehydrocurdione (**17**)



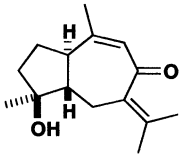
curcumenol (**18**) : R=β-CH_3
4-epicurcumenol (**19**) : R=α-CH_3



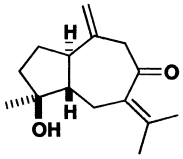
isocurcumenol (**20**)



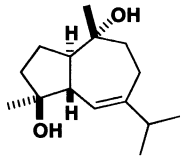
neocurcumenol (**21**)



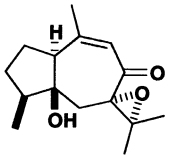
procurcumenol (**22**)



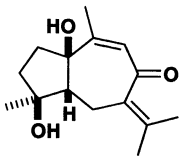
isoprocurcumenol (**23**)



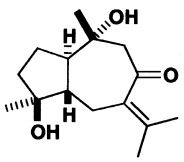
alismoxide (**24**)



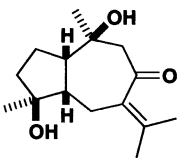
7α,11α-epoxy-5β-hydroxy-9-guaiaen-8-one (**25**)



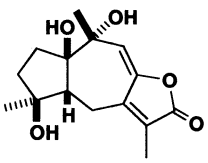
aerugidiol (**26**)



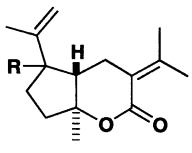
zedoarondiol (**27**)



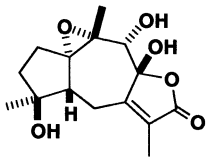
isozedoarondiol (**28**)



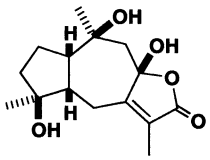
zedoalactone B (**29**)



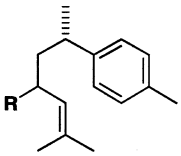
gajutsulactone A (**30**) : R=α-H
gajutsulactone B (**31**) : R=β-H



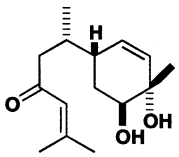
zedoarolide A (**32**)



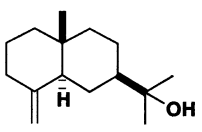
zedoarolide B (**33**)



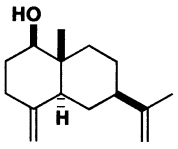
(+)-*ar*-turmerone (**34**): R= =O
bisacumol (**35**): R=α-OH



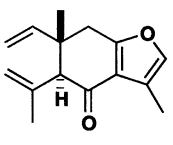
bisacurone (**36**)



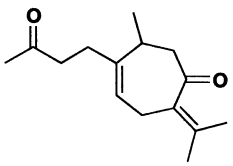
β-eudesmol (**37**)



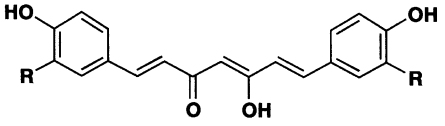
β-dictyopterol (**38**)



curzerenone (**39**)

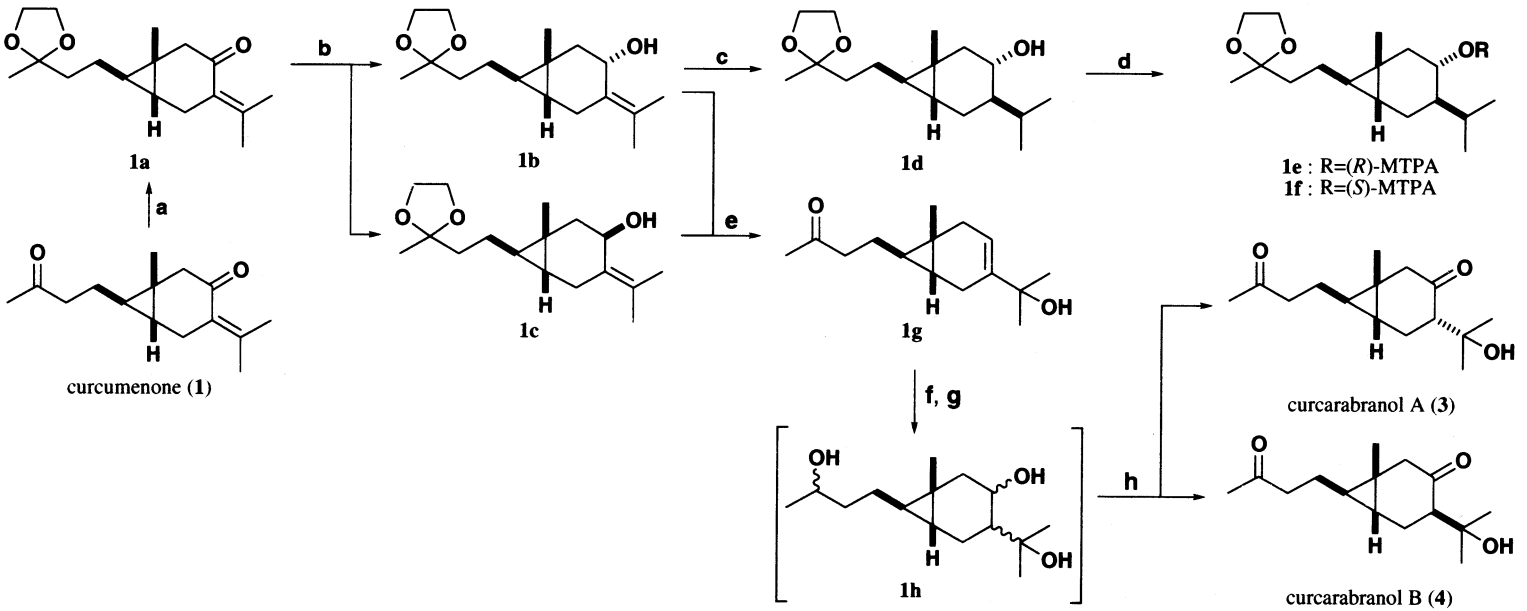


curcumadione (**40**)



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

Scheme 1.



Scheme 2. Chemical correlation of curcumenone (**1**) with curcarabranols A (**3**) and B (**4**). *Reagents and conditions*: (**a**) 1,2-bis(trimethylsilyloxy)ethane, TMSOTf, CH_2Cl_2, −78°C, 86%, (**b**) $NaBH_4$,$CeCl_3{\cdot}H_2O$, MeOH, 0°C, **1b** 68%, **1c** 23%, (**c**) $Pd(OH)_2$–C, H_2, MeOH, r.t., 45%, (**d**) (*R*)- or (*S*)-MTPA, EDC·HCl, DMAP, CH_2Cl_2, r.t., **1e** 57%, **1f** 32%, (**e**) *p*-$TsOH{\cdot}H_2O$, dioxane–H_2O [2:1 (v/v)], r.t., quant., (**f**) $BH_3{\cdot}THF$, r.t., (**g**) 30% H_2O_2, 3 M NaOH, r.t., (**h**) PCC, CH_2Cl_2, r.t., **3** 41%, **4** 14% from **1g**.

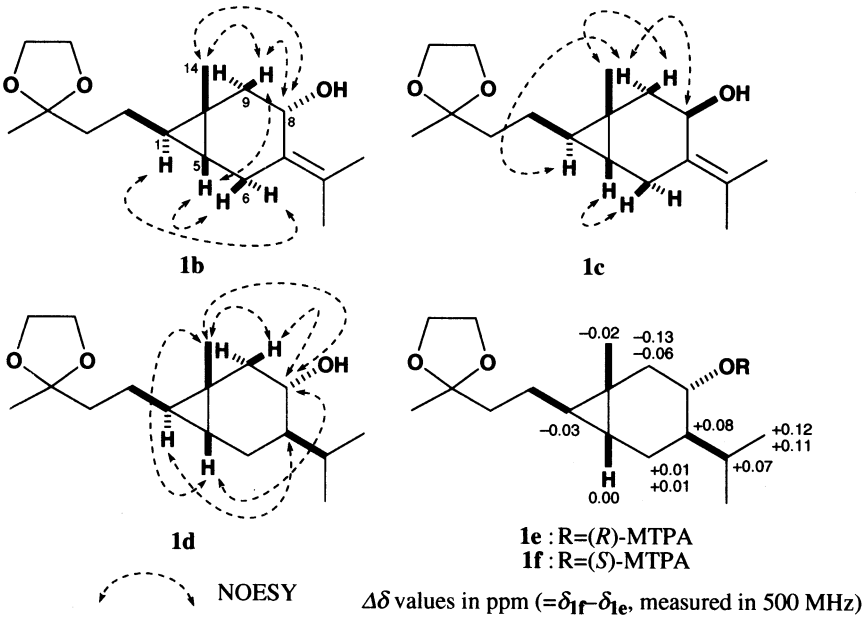


Figure 1. NOE correlations of **1a**–**1d** and application of modified Mosher's method to **1e** and **1f**.

methine bearing an oxygen function [δ 3.79 (m, 4-H)] together with four methylenes (2, 3, 6, 9-H_2) and four quaternary carbons (7, 8, 10, 11-C). The proton and carbon signals of **2** were shown to be very similar to those of a dihydrocurcumenone 4-epimeric mixture, which was obtained from the transformation products of germacrone (**10**) by cultured cells and the stereostructure was only deduced on the basis of biogenetic considerations.[6,7] The

Table 2. ^{13}C NMR data for **1a**–**1d**, **1g**, 4*S*-dihydrocurcumenone (**2**), and curcarabranols A (**3**) and B (**4**)

	1a	1b	1c	1d	1g	2	3	4
C-1	24.1	30.4	28.7	29.2	21.6	24.2	33.6	23.1
C-2	23.7	24.1	23.8	24.2	23.5	25.2	23.4	23.4
C-3	39.3	39.3	39.4	39.4	44.3	39.5	43.6	43.8
C-4	109.8	110.1	110.0	110.2	209.3	67.8	208.5	208.4
C-5	24.7	24.8	25.9	25.4	25.0	24.6	23.8	24.0
C-6	28.1	26.3	23.5	22.2	24.4	28.1	29.3	23.7
C-7	128.3	129.6	127.4	43.3	140.7	128.3	55.8	54.0
C-8	202.0	66.0	66.0	69.7	115.9	202.0	218.8	217.2
C-9	49.1	40.4	41.9	44.2	32.6	49.1	50.4	49.2
C-10	19.9	16.7	15.3	18.3	18.1	19.9	22.2	18.6
C-11	147.1	131.3	129.7	26.0	72.9	147.1	71.6	72.9
C-12	23.4[a]	20.5	15.3[a]	16.2[a]	28.7[a]	23.4	28.5[a]	28.3[a]
C-13	23.5[a]	20.5	20.5[a]	20.5[a]	28.9[a]	23.4	25.2[a]	25.0[a]
C-14	19.0	19.4	22.0	21.5	20.2	19.1	19.9	19.0
C-15	23.7	23.7	23.7	23.7	30.0	23.7	30.1	30.1
—OCH_2CH_2O—	64.6	64.6	64.6	64.6				

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

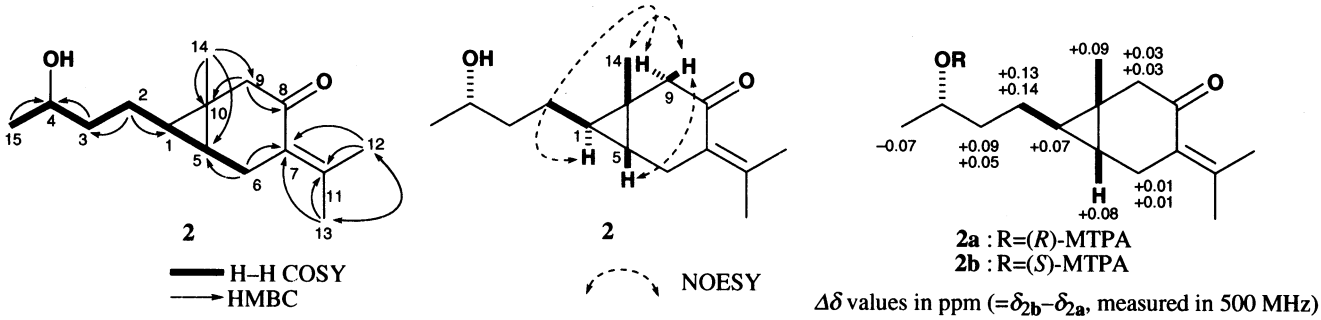
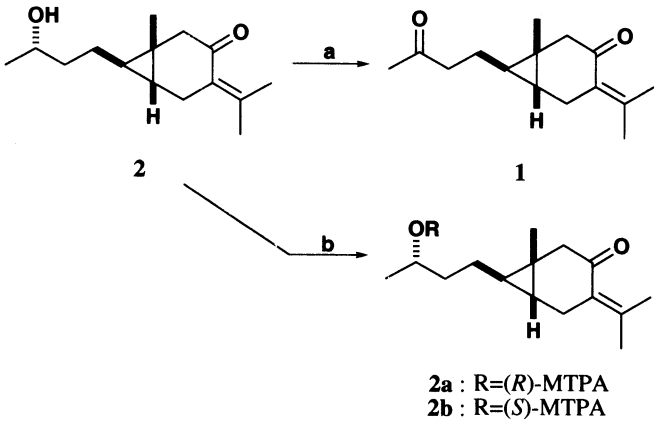


Figure 2. H–H COSY, HMBC, and NOE correlations of **2** and application of modified Mosher's method to **2a** and **2b**.



Scheme 3. *Reagents and conditions*: (**a**) CrO_3, pyridine, r.t., quant. (**b**) (*R*)- or (*S*)-MTPA, EDC·HCl, DMAP, CH_2Cl_2, r.t., **2a** 74%, **2b** 84%.

planar structure of **2** was confirmed by the $^1H-^1H$ and $^{13}C-^1H$ correlation spectroscopy (H–H and C–H COSY), and heteronuclear multiple bond correlation (HMBC) experiments as shown in Fig. 2. The stereostructure of **2** was elucidated on the basis of the NOESY experiment. Furthermore, oxidation of **2** with chromium trioxide (CrO_3) in pyridine furnished **1**, so that the absolute stereostructure of **2** was characterized except for the 4-position. Finally, the modified Mosher's method was also applied to both 4-MTPA esters **2a** and **2b** prepared from **2** (Scheme 3). As shown in Fig. 2, the proton signals due to the 1, 2, 3, 5, 6, 9, and 14-positions in the (*R*)-MTPA ester (**2a**) appeared in higher fields than those of the (*S*)-MTPA ester (**2b**), while the signals ascribable to 15-C of **2a** was observed in lower fields as compared to that of **2b**. This evidence indicated the absolute configuration at the 4-position in **2** to be *S*, and the absolute stereostructure of **2** was elucidated.

2.2. Absolute stereostructures of curcarabranols A (3) and B (4)

Curcarabranol A (**3**) was isolated as a colorless oil with negative optical rotation ($[\alpha]_D^{26}=-104.0°$). The molecular formula $C_{15}H_{24}O_3$ of **3** has been determined from the molecular ion peak at *m/z* 252 (M^+) in the EI-MS of **3** and by high-resolution MS measurement. The IR spectrum of **3** showed absorption bands ascribable to hydroxyl and carbonyl groups at 3494, 1752, and 1713 cm^{-1}. The ^1H NMR ($CDCl_3$) and ^{13}C NMR (Table 2) spectra of **3** showed signals assignable to a cyclopropane [δ 0.44 (dt, *J*=5.2, 6.0 Hz, 1-H), 0.59 (ddd, *J*=5.2, 8.2, 8.2 Hz, 5-H)], a 3-oxobutyl function [δ 1.62 (m, 2-H_2), 2.16 (s, 15-H_3), 2.52 (m, 3-H_2)], three methyls [δ 1.08, 1.20 (both s, 12, 13-H_3), 1.13 (s, 14-H_3)], and a hydroxyl group [δ 4.22 (br s, 11-OH)] together with two methylene (6, 9-H_2), a methine (7-H), and three quaternary carbons (8, 10, 11-C).

The planar structure of **3** was constructed on the basis of H–H and C–H COSY and HMBC experiments. Thus, the H–H COSY experiment on **3** indicated the presence of the partial structure (1-C–3-C and 1-C–7-C), represented by the bold line in Fig. 3. In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **3** (3-H_2, 15-H_3 and 4-C; 7-H, 9-H_2 and 8-C; 9-H_2, 14-H_3 and 10-C; 12-H_3, 13-H_3 and 11-C), so that the connectivities of the quaternary carbons (4, 8, 10, 11-C) in **3** were clarified. The above-mentioned evidence led us to confirm the skeleton of **3** to be 4,8-dioxo-11-hydroxycarabrane.

Furthermore, the relative stereostructure of **3** was characterized by the difference nuclear Overhauser effect (dif. NOE) experiment. Namely, the NOE correlations were observed between the following proton pairs: 1-H and 6α-H, 9α-H; 5-H and 6β-H, 7-H, 14-H_3; 7-H and 14-H_3; 9β-H and 14-H_3 (Fig. 3).

Curcarabranol B (**4**) was also isolated as a colorless oil with positive optical rotation ($[\alpha]_D^{26}=+77.0°$). The positive and negative-ion fast atom bombardment (FAB)-MS of **4** showed a quasimolecular ion peak at *m/z* 253 $(M+H)^+$ and *m/z* 251 $(M-H)^-$, respectively. High-resolution MS analysis revealed the molecular formula of **4** to be $C_{15}H_{24}O_3$, which was the same as that of **3**. The IR spectrum

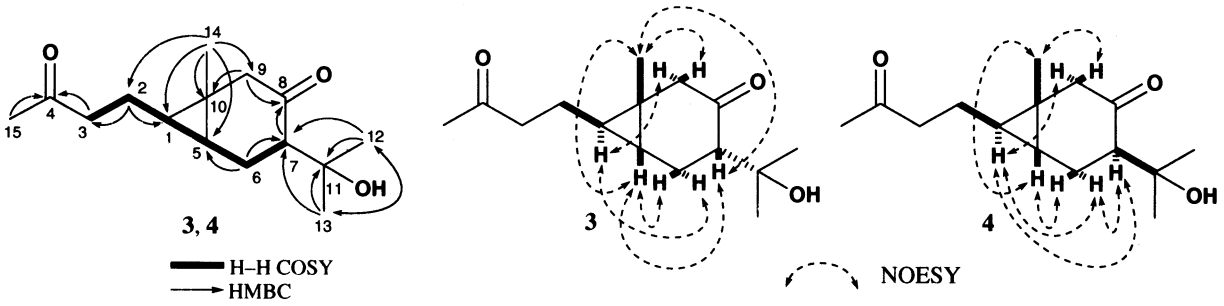


Figure 3. H–H COSY, HMBC, and NOE correlations of **3** and **4**.

Table 3. Inhibitory effects of constituents from Zedoariae Rhizoma on the contractions induced by high K^+ concentration and norepinephrine in isolated rat thoracic aorta

	Inhibition (%)				
	High K^+ (54 mM)				NE[a]
Conc.	3 μM	10 μM	30 μM	100 μM	1 μM (NE)
Sesquiterpenes					
1) Carabrane type					
Curcumenone (**1**)	2.9±0.9	5.4±1.5	12.8±2.7	12.8±2.6	−1.9±11.1
4*S*-Dihydrocurcumenone (**2**)	0.8±0.8	2.6±1.2	11.6±3.3	44.9±3.8**	−2.8±3.3
Curcarabranol A (**3**)	1.4±3.3	10.2±3.4	20.9±2.9*	39.5±4.2**	−5.4±4.0
Curcarabranol B (**4**)	0.0±1.8	7.6±2.3	20.9±1.2*	36.8±0.8**	2.5±1.4
Curcumenolactone A (**5**)	0.9±1.5	8.2±3.2	25.4±7.8**	37.7±11.7**	2.6±6.1
Curcumenolactone B (**6**)	1.8±0.8	10.8±1.6	31.7±5.3**	51.6±9.3**	−18.3±12.3
Curcumenolactone C (**7**)	0.0±0.9	3.6±1.3	8.8±1.4	16.6±1.9	−6.5±5.3
2) Germacrane type					
Furanodiene (**8**)	4.1±1.5	13.3±1.5*	33.9±3.3**	60.3±5.9**	6.8±1.5
Zederone (**10**)	4.4±0.6	10.4±1.8	29.2±3.8**	76.7±7.4**	1.5±2.8
Germacrone (**11**)	5.6±0.9*	19.3±3.0**	68.4±5.4**	94.7±1.8**	−7.3±1.7
13-Hydroxygermacrone (**12**)	3.2±1.4	7.7±3.9	26.1±3.3*	74.7±2.2**	−3.2±3.2
Glechomanolide (**13**)	−0.5±1.0	9.3±4.8	36.8±9.5	92.0±6.3**	12.9±10.0
(+)-Germacrone 4,5-epoxide (**14**)	−0.2±2.0	4.8±5.0	17.3±6.8	47.6±6.3**	−3.0±11.8
Curdione (**15**)	9.8±4.3	14.8±4.8	23.7±5.4*	44.2±7.2**	3.6±6.5
Neocurdione (**16**)	−0.3±1.8	12.5±6.0	30.4±10.4**	69.7±12.9**	−17.7±8.9
Dehydrocurdione (**17**)	2.7±0.8	6.1±1.2	13.9±2.0	30.3±3.0**	−10.3±4.2
3) Guaiane type					
Curcumenol (**18**)	0.6±0.4	1.5±0.6	18.5±2.7*	54.5±5.9**	−4.2±1.5
4-Epicurcumenol (**19**)	2.9±1.9	0.3±1.2	6.9±3.9	52.1±5.7**	19.6±5.5
Isocurcumenol (**20**)	4.1±0.5	13.8±3.0**	55.3±6.4**	88.5±2.5**	−7.8±3.5
Neocurcumenol (**21**)	0.1±0.9	2.1±1.5	14.2±3.2	58.7±5.6**	2.9±3.4
Procurcumenol (**22**)	4.7±1.8	11.8±3.7	20.4±7.0	31.9±9.3*	13.7±0.3
Isoprocurcumenol (**23**)	−2.4±0.9	7.5±3.9	16.9±5.7	29.8±5.8*	25.6±8.2
Alismoxide (**24**)	3.8±1.6	11.0±3.1	23.1±2.6**	40.0±2.7**	6.5±6.9
7α,11α-Epoxy-5β-hydroxy-9-guaiaen-8-one (**25**)	1.7±1.1	8.1±0.6	19.0±2.3*	43.3±3.8**	−9.9±5.7
Aerugidiol (**26**)	0.4±0.4	1.1±1.6	1.4±0.8	2.7±0.9	2.3±4.3
Zedoarondiol (**27**)	4.8±0.7	9.2±1.7	11.5±2.9	16.3±3.9*	5.6±4.3
Isozedoarondiol (**28**)	2.5±1.5	4.7±2.7	6.0±3.5	8.4±3.9	−1.6±3.0
Zedoalactone B (**29**)	6.9±1.9	12.7±3.0*	17.1±3.4*	21.6±3.8**	−2.8±1.3
Zedoarolide A (**32**)	9.2±1.7*	19.7±3.0*	29.1±5.2**	42.0±5.8**	1.7±1.3
Zedoarolide B (**33**)	−3.4±1.5	−1.6±3.3	4.5±3.8	13.5±2.7	−3.5±5.3
4) Bisaborane type					
(+)-*ar*-Turumerone (**34**)	3.7±0.7	11.0±2.1*	37.2±6.3**	78.3±6.4**	9.8±6.5
Bisacumol (**35**)	−0.8±0.5	6.5±2.1	56.5±6.2**	75.5±8.1**	−12.2±3.6
Bisacurone (**36**)	−4.7±1.1	−5.4±2.5	0.4±2.0	5.0±1.9	−8.7±6.7
5) Eudesmane type					
β-Eudesmol (**37**)	5.1±1.4	29.0±4.1**	90.9±3.7**	95.0±2.5**	−0.2±12.5
β-Dictyopterol (**38**)	10.8±3.4**	53.1±5.5**	93.1±1.4**	88.1±3.3**	9.3±7.2
6) Elemane type					
Curzerenone (**39**)	3.5±1.1	14.9±1.9*	38.0±5.5**	79.0±8.7**	−10.3±4.2
7) Xanthane type					
Curcumadione (**40**)	1.9±1.0	7.3±1.5	14.3±1.5	27.5±2.1**	−15.2±7.5
Diarylheptanoids					
Curcumin (**41**)	6.8±2.8	23.3±7.5	46.1±8.8**	75.7±8.9**	37.7±12.5**
Bis(4-hydroxycinnamoyl)-methane (**42**)	9.3±3.5*	22.9±3.4**	62.7±7.6**	87.9±3.8**	38.4±2.3**

Each value represents the mean±SEM (n=4–6). Significantly different from the control: $^*p<0.05$, $^{**}p<0.01$.
[a] Sample concentrations were 10^{-4} M.

of **4** showed absorption bands at 3494, 1762, and 1709 cm^{-1} due to hydroxyl and carbonyl functions. The proton and carbon signals in the ^1H NMR ($CDCl_3$) and ^{13}C NMR spectra (Table 2) of **4** were shown to be very similar to those of **3**; a cyclopropane [δ 0.55 (dt, J=6.9, 7.3 Hz, 1-H), 0.72 (m, 5-H)], a 3-oxobutyl function [δ 1.63 (dt, J=7.3, 7.3 Hz, 2-H_2), 2.15 (s, 15-H_3), 2.48 (m, 3-H_2)], three methyls [δ 1.11 (s, 14-H_3), 1.12, 1.17 (both s, 12, 13-H_3)], and a hydroxyl group [δ 4.49 (br s, 11-OH)]. The correlation and connectivities of **4** were observed between the protons and quaternary carbons were determined on the basis of H–H, C–H COSY and HMBC experiments, and the relative structure with the 7β-isopropyl group was also elucidated by NOE correlations (1-H and 6α-H, 7-H, 9α-H; 5-H and 6β-H, 14-H_3; 6α-H and 7-H; 9β-H and 14-H_3); thus **4** was characterized to be the 7-position isomer of **3**.

Next, in order to clarify the absolute stereostructures of curcarabranols A (**3**) and B (**4**), we carried out the

conversion of curcumenone (**1**) into **3** and **4**. At first, acid treatment of **1b** or **1c** with *p*-toluenesulfonic acid (*p*-TsOH·H_2O) furnished **1g**, in which the hydroxyl group migrated from the 8-position to the 11-position and the ketal group was removed. Then, **1g** was treated with borane–tetrahydrofuran complex (BH_3·THF), followed by H_2O_2 oxidation to yield a complex mixture of the triol (**1h**), which was subsequently subjected to oxidation with pyridinium chlorochromate (PCC) to give **3** (41% from **1g**) and **4** (14% from **1g**) in an approximate 3:1 ratio. On the basis of the chemical transformation from **1** to **3** and **4**, the absolute stereostructures of curcarabranols A (**3**) and B (**4**) were determined as shown.

2.3. Vasorelaxant effects of isolated constituents from Zedoariae Rhizoma

As shown in Table 3, 16 sesquiterpenes [curcumenolactone B (**6**, IC_{50}=100 μM), furanodiene (**8**, 67 μM), zederone (**10**, 46 μM), germacrone (**11**, 19 μM), 13-hydroxygermacrone (**12**, 52 μM), glechomanolide (**13**, 35 μM), neocurdione (**16**, 54 μM), curcumenol (**18**, 92 μM), 4-epicurcumenol (**19**, 90 μM), isocurcumenol (**20**, 26 μM), neocurcumenol (**21**, 85 μM), (+)-*ar*-turumerone (**34**, 38 μM), bisacumol (**35**, 37 μM), β-eudesmol (**37**, 16μM), β-dictyopterol (**38**, 9 μM), and curzerenone (**39**, 38 μM)] and two diarylheptanoids [curcumin (**41**, 32 μM) and bis-(4-hydroxycinnamoyl)methane (**42**, 22 μM)] relaxed the sustained contractions induced by high concentrations of K^+. Especially, five sesquiterpenes (**11**, **13**, **20**, **37**, **38**) showed potent relaxation, and these results suggested that activities of germacrane- and eudesmane-type sesquiterpenes were stronger than those of the other types of sesquiterpene. Furthermore, compounds having an *exo*-methylene moiety, such as **20**, and **37**–**39**, showed potent activity. On the other hand, polyoxygenated sesquiterpenes such as **26**–**29**, **33**, and **36** showed weak activity. These results led us to presume the following structural requirements of the isolated sesquiterpenes for activity:

1. germacrane- and eudesmane-type sesquiterpenes were active structures;
2. the *exo*-methylene moiety enhanced the activity; and
3. polyoxygenated sesquiterpenes exhibited weak activity.

In addition, inhibitory effects of the isolated constituents on NE-induced contractions were examined in isolated thoracic aorta of rat. As a result, diarylheptanoids (**41**, **42**) slightly inhibited the contractions. However, other sesquiterpene constituents did not show the significant inhibition as shown in Table 3.

It is well known that high K^+-induced contractions in smooth muscles are the result of an increase in intracellular Ca^{2+}, and calcium antagonists such as nifedipine inhibit the voltage-dependent calcium channel, thereby inhibiting the contractions in the depolarized aortic strips, but they show weak inhibitory effects on the NE-induced contractions.[12] The results in the present experiments suggested that curcumenolactone B (**6**), furanodiene (**8**), zederone (**10**), germacrone (**11**), 13-hydroxygermacrone (**12**), glechomanolide (**13**), neocurdione (**16**), curcumenol (**18**), 4-epicurcumenol (**19**), isocurcumenol (**20**), neocurcumenol (**21**), (+)-*ar*-turumerone (**34**), bisacumol (**35**), β-eudesmol (**37**), β-dicytopterol (**38**), and curzerenone (**39**) relaxed high K^+-induced contractions by their calcium channel-blocking activities like nifedipine. The vasorelaxant effect of these active sesquiterpenes may be related to the traditional medicinal value of Zedoariae Rhizoma as the treatment effect of the 'Oketsu' syndrome caused by blood stagnation.

3. Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H NMR spectra, JNM-LA500 (500 MHz); ^{13}C NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer, JEOL JMS-SX 102A mass spectrometer.

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

3.1. Extraction and isolation

Curcumenone (**1**), 4*S*-dihydrocurcumenone (**2**), and curcarabranols A (**3**) and B (**4**) were isolated from the dried Zedoariae Rhizoma cultivated in Szechwan, China as described earlier.[3]

3.1.1. 4*S*-Dihydrocurcumenone (2). Colorless oil, $[\alpha]_D^{27}$=−5.1° (c=0.1, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{24}O_2$ (M^+): 236.1776. Found: 236.1767. UV [EtOH, nm (log ε)]: 254 (2.90). IR (film): 3436, 2924, 1678, 1599, 1053 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.46 (1H, dt, J=4.9, 6.4 Hz, 1-H), 0.65 (1H, m, 5-H), 1.12 (3H, s, 14-H_3), 1.18 (3H, d, J=6.1 Hz, 15-H_3), 1.33 (1H, m, 2-H), 1.46–1.50 (3H, m, 2-H and 3-H_2), 1.79, 2.09 (3H each, both s, 12 and 13-H_3), 2.52, 2.56 (2H, ABq, J=15.6 Hz, 9-H_2), 2.82 (2H, br s, 6-H_2), 3.79 (1H, m, 4-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. EI-MS: m/z 236 (M^+, 15), 218 (M^+–H_2O, 20), 68 (100).

3.1.2. Curcarabranol A (3). Colorless oil, $[\alpha]_D^{26}$=−104.0° (c=0.1, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{24}O_3$ (M^+): 252.1726. Found: 252.1741. IR (film): 3494, 2930, 1752, 1713, 1020 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.44 (1H, dt, J=5.2, 6.0 Hz, 1-H), 0.59 (1H, ddd, J=5.2, 8.2, 8.2 Hz, 5-H), 1.08, 1.20 (3H each, both s, 12 and 13-H_3), 1.13 (3H, s, 14-H_3), 1.46 (1H, m, 6α-H), 1.62 (2H, m, 2-H_2), 2.16 (3H, s, 15-H_3), 2.22, 2.52 (2H, ABq, J=15.0 Hz, 9-H_2), 2.37 (1H, dd, J=5.5, 7.0 Hz, 7-H), 2.52

(2H, m, 3-H_2), 2.54 (1H, m, 6β-H), 4.22 (1H, br s, 11-OH). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. EI-MS: *m/z* 252 (M^+, 5), 234 (M^+-H_2O, 25), 43 (100).

3.1.3. Curcarabranol B (4). Colorless oil, $[\alpha]_D^{26}=+77.0°$ (c=0.1, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{15}H_{25}O_3$ $(M+H)^+$: 253.1804. Found: 253.1818. IR (film): 3494, 2973, 1762, 1709, 1025 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.55 (1H, dt, J=6.9, 7.3 Hz, 1-H), 0.72 (1H, m, 5-H), 1.11 (3H, s, 14-H_3), 1.12, 1.17 (3H each, both s, 12 and 13-H_3), 1.63 (2H, dt, J=7.3, 7.3 Hz, 2-H_2), 2.00 (1H, m, 6β-H), 2.07 (1H, m, 7-H), 2.15 (3H, s, 15-H_3), 2.20 (1H, m, 6α-H), 2.46, 2.61 (2H, ABq, J=17.1 Hz, 9-H_2), 2.48 (2H, m, 3-H_2), 4.49 (1H, br s, 11-OH). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. Positive-ion FAB-MS: *m/z* 253 $(M+H)^+$. Negative-ion FAB-MS: *m/z* 251 $(M-H)^-$.

3.2. Ketalization of curcumenone (1)

A solution of **1** (30.0 mg, 128 μmol) in CH_2Cl_2 (2.0 ml) was treated with 1,2-bis(trimethylsilyloxy)ethane (38 μl) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst and the whole mixture was stirred at −78°C for 14 h. The reaction mixture was quenched in pyridine, then poured into saturated aqueous $NaHCO_3$ and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with saturated aqueous NaCl and dried over a 1:1 mixture of K_2CO_3 and $MgSO_4$ powder, then the mixture was filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [2.0 g, *n*-hexane–AcOEt=10:1] to give **1a** (30.6 mg, 86%).

1a: Colorless oil. High-resolution EI-MS: calcd for $C_{17}H_{26}O_3$ (M^+): 278.1882. Found: 278.1882. IR (film): 2932, 1752, 1680, 1061, 1097, 1061 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.47 (1H, dt, J=4.6, 7.0 Hz, 1-H), 0.65 (1H, br dd, J=ca. 5, 7 Hz, 5-H), 1.12, 1.29 (3H each, both s, 14 and 15-H_3), 1.41 (2H, m, 2-H_2), 1.68 (2H, t-like, 3-H_2), 1.79, 2.09 (3H each, both s, 12 and 13-H_3), 2.51, 2.56 (2H, ABq, J=15.8 Hz, 9-H_2), 2.82 (2H, br s, 6-H_2), 3.92 (4H, m, —O—CH_2CH_2—O—). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. EI-MS: *m/z* 278 (M^+, 5), 87 (100).

3.3. $NaBH_4$–$CeCl_3$ reduction of 1a

A solution of **1a** (24.2 mg, 87 μmol) in MeOH (1.0 ml) was treated with $NaBH_4$ (13.2 mg, 349 μmol) in the presence of $CeCl_3{\cdot}H_2O$ (10.0 mg) and the mixture was stirred at 0°C for 20 min. The reaction mixture was quenched in acetone, then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine and then dried over $MgSO_4$ powder. Removal of the solvent under reduced pressure yielded a residue, which was purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O=80:20 v/v] to give **1b** (16.5 mg, 68%) and **1c** (5.6 mg, 23%).

1b: Colorless oil, $[\alpha]_D^{24}=+59.1°$ (c=0.2, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{17}H_{28}O_3$ (M^+): 280.2065. Found: 280.2070. IR (film): 3475, 2932, 1762, 1651, 1100, 1069 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.32 (1H, br dd, J=ca. 5, 6 Hz, 5-H), 0.84 (1H, dt, J=4.9, 6.4 Hz, 1-H), 1.03, 1.31 (3H each, both s, 14 and 15-H_3), 1.39 (2H, m, 2-H_2), 1.55 (1H, dd, J=6.1, 14.8 Hz, 9α-H), 1.68, 1.79 (3H each, both s, 12 and 13-H_3), 1.73 (2H, t, J=8.8 Hz, 3-H_2), 1.99 (1H, dd, J=6.1, 14.8 Hz, 9β-H), 2.03 (1H, m, 6α-H), 2.69 (1H, dd, J=6.4, 10.2 Hz, 6β-H), 3.92 (4H, m, —O—CH_2CH_2—O—), 4.61 (1H, dd, J=6.1, 6.1 Hz, 8-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. EI-MS: *m/z* 280 (M^+, 2), 262 (M^+-H_2O, 10), 87 (100).

1c: Colorless oil, $[\alpha]_D^{24}=-51.8°$ (c=0.1, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{17}H_{28}O_3$ (M^+): 280.2065. Found: 280.2039. IR (film): 3475, 2921, 1762, 1651, 1093, 1063 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.10 (1H, dt, J=5.2, 7.3 Hz, 1-H), 0.54 (1H, br dd, J=ca. 5, 8 Hz, 5-H), 1.13, 1.31 (3H each, both s, 14 and 15-H_3), 1.39 (2H, m, 2-H_2), 1.55, 1.64 (3H each, both s, 12 and 13-H_3), 1.69 (2H, t, J=6.7 Hz, 3-H_2), 1.71 (1H, dd, J=4.0, 14.7 Hz, 9α-H), 1.95 (1H, dd, J=4.0, 14.7 Hz, 9β-H), 2.50 (1H, dd-like, 6β-H), 2.59 (1H, m, 6α-H), 3.93 (4H, m, —O—CH_2CH_2—O—), 4.57 (1H, dd, J=4.0, 4.0 Hz, 8-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. EI-MS: *m/z* 280 (M^+, 5), 262 (M^+-H_2O, 10), 87 (100).

3.4. Hydrogenation of 1b

A solution of **1b** (9.0 mg, 32 μmol) in MeOH (0.5 ml) was treated with 10% palladium hydroxide carbon [$Pd(OH)_2$–C, 10.0 mg] and the whole mixture was stirred at room temperature under a H_2 atmosphere for 3 h. The catalyst was filtered off, and the solvent from the filtrate was evaporated under reduced pressure to give a residue, which was purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O (80:20, v/v)] to give **1d** (4.0 mg, 45%).

1d: Colorless oil, $[\alpha]_D^{23}=+48.1°$ (c=0.1, $CHCl_3$). High-resolution positive-ion FAB-MS: Calcd for $C_{17}H_{30}O_3Na$ $(M+Na)^+$: 305.2093. Found: 305.2121. IR (film): 3450, 2955, 2872, 1743, 1464, 1219, 1063 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.32 (1H, ddd, J=5.4, 6.1, 7.0 Hz, 5-H), 0.40 (1H, dt, J=5.4, 7.0 Hz, 1-H), 0.82, 0.88 (3H each, both d, J=7.0 Hz, 12 and 13-H_3), 1.01 (1H, m, 7-H), 1.06, 1.31 (3H each, both s, 14 and 15-H_3), 1.39 (1H, dd, J=10.0, 11.3 Hz, 9α-H), 1.40 (2H, m, 2-H_2), 1.68, 1.71 (1H each, both m, 3-H_2), 1.75 (2H, m, 6-H_2), 2.06 (1H, dd, J=4.8, 11.3 Hz, 9β-H), 2.11 (1H, dq, J=3.6, 7.0 Hz, 11-H), 3.40 (1H, ddd, J=4.8, 5.5, 10.0 Hz, 8-H), 3.94 (4H, m, —O—CH_2CH_2—O—). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. Positive-ion FAB-MS: *m/z* 305 $(M+Na)^+$.

3.5. Preparation of the (*R*)-MTPA ester (1e) and the (*S*)-MTPA ester (1f) from 1d

A solution of **1d** (2.0 mg, 7 μmol) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-MTPA (16.6 mg, 70 μmol) in the presence of EDC·HCl (14.6 mg, 70 μmol) and 4-DMAP (5.2 mg, 42 μmol) and the mixture was stirred at room temperature for 18 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. Removal of the solvent under reduced pressure furnished a residue, which was purified by silica

gel column chromatography (300 mg, *n*-hexane–AcOEt=5:1) to give **1e** (2.0 mg, 57%).

By the same procedure as used for **1e**, the (*S*)-MTPA ester (**1f**, 1.1 mg, 32%) was prepared from **1d** (2.0 mg) using (*S*)-MTPA (16.6 mg, 70 μmol), EDC·HCl (14.6 mg, 70 μmol) and 4-DMAP (5.2 mg, 42 μmol).

1e: Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.38 (1H, ddd, *J*=4.8, 5.2, 6.1 Hz, 5-H), 0.43 (1H, dt, *J*=6.1, 6.7 Hz, 1-H), 0.64, 0.73 (3H each, both d, *J*=7.0 Hz, 12 and 13-H_3), 1.09, 1.31 (3H each, both s, 14 and 15-H_3), 1.26 (1H, m, 7-H), 1.38 (2H, m, 2-H_2), 1.55, 2.18 (1H each, both m, 9-H_2), 1.64 (2H, m, 6-H_2), 1.74 (2H, m, 3-H_2), 1.75 (1H, m, 11-H), 3.57 (3H, s, —OCH_3), 3.92 (4H, m, —O—CH_2CH_2—O—), 4.88 (1H, ddd, *J*=4.3, 5.8, 10.1 Hz, 8-H), 7.32–7.55 (5H, m, ph-H).

1f: Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.38 (1H, m, 5-H), 0.40 (1H, m, 1-H), 0.76, 0.84 (3H each, both d, *J*=7.0 Hz, 12 and 13-H_3), 1.07, 1.31 (3H each, both s, 14 and 15-H_3), 1.33 (1H, m, 7-H), 1.37 (2H, m, 2-H_2), [1.42 (1H, dd, *J*=9.5, 13.4 Hz), 2.12 (1H, dd, *J*=6.1, 13.4 Hz), 9-H_2], 1.65 (2H, m, 6-H_2), 1.74 (2H, m, 3-H_2), 1.83 (1H, m, 11-H), 3.56 (3H, s, —OCH_3), 3.92 (4H, m, —O—CH_2CH_2—O—), 4.88 (1H, ddd, *J*=4.4, 6.1, 9.5 Hz, 8-H), 7.33–7.54 (5H, m, ph-H).

3.6. CrO_3–pyridine oxidation of 4*S*-dihydrocurcumenone (2)

A solution of **2** (2.4 mg, 10 μmol) in pyridine (0.5 mg) was treated with CrO_3 (3.0 mg)–pyridine (0.5 ml) mixture, and the whole mixture was stirred at room temperature for 1 h. The reaction mixture was poured into saturated aqueous NaCl and the whole was extracted with AcOEt. The AcOEt extract was washed with brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a crude product which was purified by silica gel column chromatography (200 mg, *n*-hexane–AcOEt=5:1) to furnish **1** (2.3 mg, quant.).

3.7. Preparation of the (*R*)-MTPA ester (2a) and the (*S*)-MTPA ester (2a) from 4*S*-dihydrocurcumenone (2)

A solution of **2** (2.6 mg, 11 μmol) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-MTPA (16.6 mg, 70 μmol) in the presence of EDC·HCl (14.6 mg, 70 μmol) and 4-DMAP (5.2 mg, 42 μmol) and the mixture was stirred at room temperature for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above gave a product, which was purified by silica gel column chromatography (300 mg, *n*-hexane–AcOEt=10:1) to give **2a** (3.7 mg, 74%).

By the same procedure as used for **2a**, the compound **2b** (4.5 mg, 84%) was prepared from **2** (2.8 mg, 11 μmol) using (*S*)-MTPA (16.6 mg, 70 μmol), EDC·HCl (14.6 mg, 70 μmol) and 4-DMAP (5.2 mg, 42 μmol).

2a: Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.35 (1H, dt, *J*=4.9, 6.8 Hz, 1-H), 0.51 (1H, m, 5-H), 0.97 (3H, s, 14-H_3), 1.15, 1.29 (1H each, both m, 2-H_2), 1.32 (3H, d, *J*=6.1 Hz, 15-H_3), 1.56, 1.64 (1H each, both m, 3-H_2), 1.80, 2.09 (3H each, both s, 12 and 13-H_3), 2.47, 2.52 (2H, ABq, *J*=15.2 Hz, 9-H_2), 2.81 (2H, br s, 6-H_2), 3.56 (3H, s, —OCH_3), 5.12 (1H, m, 4-H), [7.39 (2H, m), 7.53 (3H, m), ph-H].

2b: Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.42 (1H, dt, *J*=4.6, 7.3 Hz, 1-H), 0.59 (1H, br dd, *J*=ca. 5, 8 Hz, 5-H), 1.06 (3H, s, 14-H_3), 1.25 (3H, d, *J*=6.1 Hz, 15-H_3), 1.28, 1.43 (1H each, both m, 2-H_2), 1.61, 1.73 (2H, m, 3-H_2), 1.80, 2.09 (3H each, both s, 12 and 13-H_3), 2.50, 2.55 (2H, ABq, *J*=15.5 Hz, 9-H_2), 2.82 (2H, br s, 6-H_2), 3.56 (3H, s, —OCH_3), 5.12 (1H, m, 4-H), [7.40 (2H, m), 7.47 (3H, m), ph-H].

3.8. Acid treatment with 1b and 1c

A solution of **1b** (13.6 mg, 48 μmol) in 1,4-dioxane–H_2O (2: 1 v/v, 1.5 ml) was treated with *p*-TsOH·H_2O (10 mg) and the whole mixture was stirred at room temperature for 3 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$ powder. Removal of the solvent under reduced pressure furnished a residue, which was purified by silica gel column chromatography (500 mg, *n*-hexane–AcOEt=5:1) to give **1g** (11.5 mg, quant.). Through a similar procedure, **1g** (4.3 mg, quant.) was also prepared from **1c** (5.0 mg, 18 μmol).

1g: Colorless oil, $[\alpha]_D^{25}=-4.6°$ (*c*=0.1, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{15}H_{22}O$ (M^+-H_2O): 218.1654. Found: 218.1671. IR (film): 3650, 2939, 1717, 1700, 1031 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.53 (1H, dt, *J*=4.9, 7.0 Hz, 1-H), 0.56 (1H, ddd, *J*=4.9, 4.9, 6.7 Hz, 5-H), 1.09 (3H, s, 14-H_3), 1.27, 1.27 (3H each, both s, 12 and 13-H_3), 1.62 (2H, m, 2-H_2), 2.12, 2.29 (1H each, both m, 9-H_2), 2.14 (3H, s, 15-H_3), 2.32, 2.38 (1H, each, both m, 6-H_2), 2.46 (2H, t, *J*=7.6 Hz, 3-H_2), 5.51 (1H, dd, *J*=2.5, 5.2 Hz, 8-H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. EI-MS: *m*/*z* 218 (M^+-H_2O, 10), 160 (100).

3.9. Conversion from 1g to 3 and 4

A solution of **1g** (15.0 mg, 64 μmol) in THF (1.0 ml) was treated with 1.0 M BH_3·THF (0.5 ml) and the whole mixture was stirred at room temperature for 2 h. The reaction mixture was quenched in ice-water, then the solution was treated with 3.0 M NaOH (1.0 ml) and 30% aqueous H_2O_2 (1.0 ml) at room temperature. After 2 h, the whole mixture was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$ powder, then the mixture was filtered. Removal of the solvent from the filtrate under reduced pressure furnished the triol mixture (**1h**). A solution of the triol mixture (**1h**) in CH_2Cl_2 (1.0 ml) was successively treated with PCC (20.0 mg) and the whole mixture was stirred at room temperature for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a crude product, which was

purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O (50: 50, v/v)] to furnish **3** (6.6 mg, 41%) and **4** (2.2 mg, 14%).

3.10. Bioassay

3.10.1. Tissue preparation. Male Wistar rats weighing 250–350 g were sacrificed by severing both carotid arteries under anesthesia, and the thoracic aorta was isolated and cut into helical strips (2–3 mm×15–20 mm). Physiological salt solution contained NaCl (118.0 mM), KCl (4.7 mM), KH_2PO_4 (1.2 mM), $MgSO_4$ (1.2 mM), $CaCl_2$ (2.5 mM), $NaHCO_3$ (25.0 mM), and D-glucose (10.0 mM). The solution was aerated with a 95% O_2–5% CO_2 gas mixture and kept at 37°C. To investigate the mechanical response, each preparation was suspended in an organ bath (6 ml) and subjected to an initial load of about 1 g. One hour equilibration period was allowed before initiation of the experiments. Contractions were measured isometrically via a force-displacement transducer (Nihon Denki Sanei, Tokyo, Japan) and recorded on a polygraph.

3.10.2. High K^+-induced contraction. After equilibration, 3 M KCl (0.1 ml) was added to the bath (final concentration of K^+: 54 mM). The tissues were washed three times and re-equilibrated after the contraction had reached the maximum level. Sustained contraction was induced again by the addition of KCl, and then test compound was cumulatively applied at 3–100 μM. The contractile response prior to the application of the test sample was taken to be 100%. Nifedipine was used as a reference compound.

3.10.3. NE-induced contraction. After equilibration, NE was added to the bath (final concentration of NE: 1 μM) in the presence of nifedipine (1 μM, a voltage-dependent Ca^{2+}-channel blocker). The tissues were washed three times and re-equilibrated after the contraction had reached the maximum level. This procedure was repeated, and a second contraction was obtained. Tissues were incubated with each test compound for 10 min and then NE was cumulatively applied (1 nM –1 μM). In order to minimize variability between tissues, the contraction ratio of the third response to the second response at 1 μM NE was used. The mean contraction ratio in the control was taken to be 100%. Prazosin hydrochloride was used as a reference compound. Table 3 shows the inhibition (%) of each constituent (100 μM) at 1 μM NE.

3.10.4. Statistical analysis. Values are expressed as the means±SEM. For statistical analysis, one-way analysis of variance following Dunnett's test was used. IC_{50} values were determined graphically.

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