Absolute Stereostructures of Carabrane-Type Sesquiterpenes, Curcumenone, 4*S*-Dihydrocurcumenone, and Curcarabranols A and B: Vasorelaxant Activity of Zedoary Sesquiterpenes

Masayuki YOSHIKAWA,* Toshiyuki MURAKAMI, Toshio MORIKAWA, and Hisashi MATSUDA

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.
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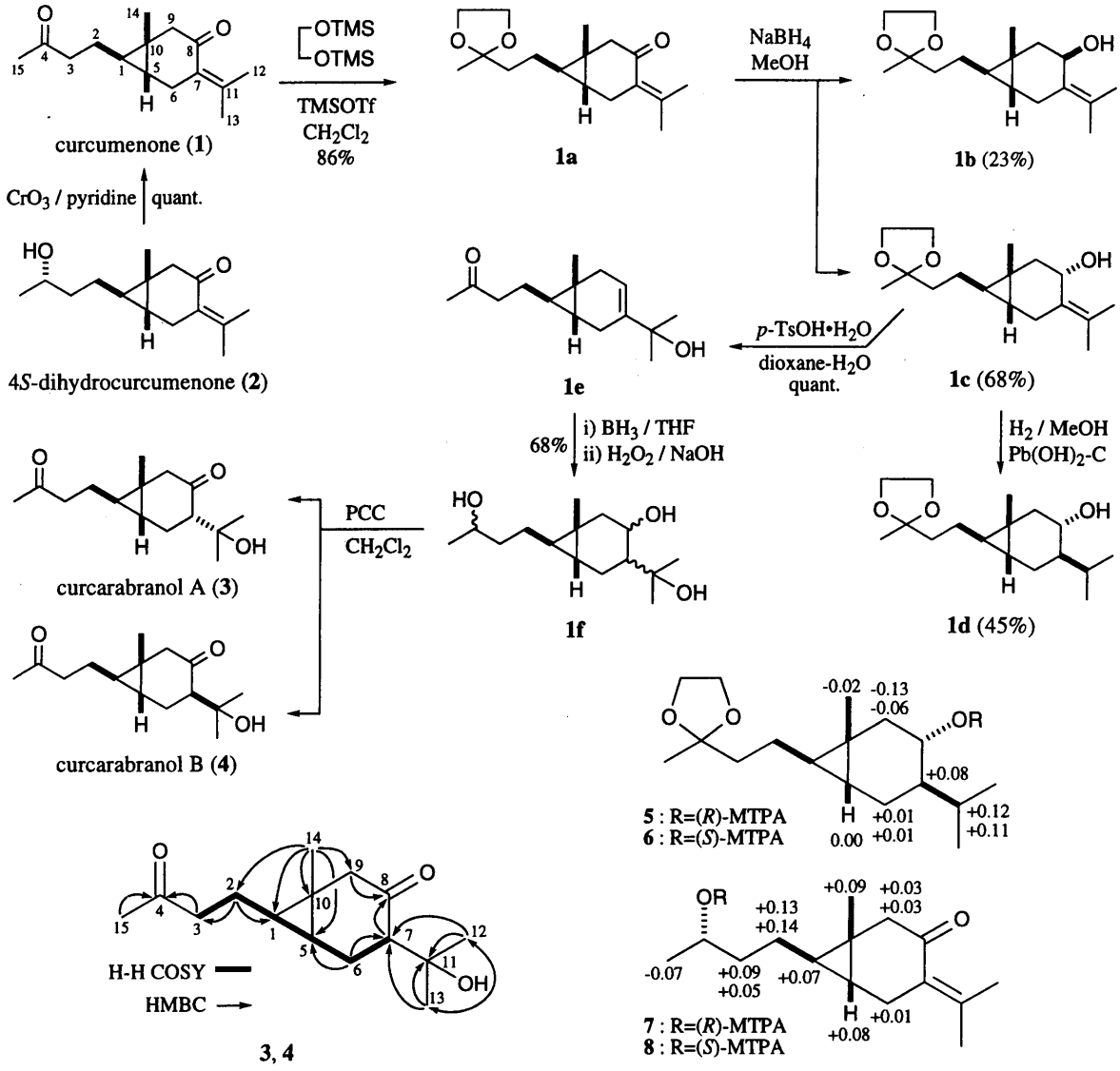
New carabrane-type sesquiterpenes, curcarabranols A and B, were isolated from zedoary together with the two known carabrane-type sesquiterpenes curcumenone and 4*S*-dihydrocurcumenone. The absolute stereostructures of these carabrane-type sesquiterpenes were determined on the basis of physicochemical and chemical evidence. Zedoary sesquiterpenes including 4*S*-dihydrocurcumenone and curcarabranols A and B were found to inhibit the contraction induced by a high concentration of K^+ in isolated rat aortic strips.

Key words carabrane-type sesquiterpene; absolute stereostructure; curcumenone; curcarabranol; vasorelaxant effect; zedoary

Zedoary (Zedoariae rhizoma) has been widely cultivated as a vegetable or spice in Asian countries. In Chinese traditional medicine, zedoary has been prescribed as a stomachic, emmenagogue, or for the treatment of "Oketsu" syndrome caused by blood stagnation in various preparations. During the course of our characterization studies on the bioactive constituents of medicinal foodstuffs[1] and natural medicines,[2] we have found that



* To whom correspondence should be addressed.

the aq. acetone extract of Chinese zedoary exhibited hepatoprotective and vasorelaxant activities. From the aq. acetone extract, several known constituents with hepatoprotective activity were identified and their action mechanisms were reported.[3] Recently, we have isolated new carabrane-type sesquiterpenes called curcarabranols A (**3**) and B (**4**) together with the two known carabrane-type sesquiterpenes curcumenone (**1**)[4] and 4*S*-dihydrocurcumenone (**2**).[5] Thus far several carabrane-type sesquiterpenes have been isolated and their structures reported, but their absolute stereostructures remained uncharacterized.[5] This communication deals with the elucidation of the absolute stereostructures of carabrane-type sesquiterpenes (**1**—**4**) and the vasorelaxant effect of zedoary sesquiterpenes.

The ethyl acetate-soluble and butanol-soluble portions from the aq. acetone extract of Chinese zedoary was subjected to silica gel, silver nitrate-treated silica gel, and ODS column chromatography and finally HPLC to provide **1** (0.0410% from zedoary), **2** (0.0011%), **3** (0.0003%), and **4** (0.0003%) together with 27 sesquiterpenes.

The absolute stereostructure of curcumenone (**1**), which was previously isolated from zedoary and its relative stereostructure clarified by NOE experiment,[4] was determined by the application of the modified Mosher's method[6] to the 8α-hydroxyl dihydro-derivative (**1d**). Thus **1** was subjected to ketalization to give **1a**,[7] which was treated with $NaBH_4$ to yield the 8β-hydroxyl (**1b**) and the 8α-hydroxyl (**1c**) derivatives. Hydogenation of **1c** furnished **1d**[8] and its stereostructure was clarified based on the difference NOE experiment, which showed NOE correlations between the 7α-H and the 1α-H and between the 8β-H and the 5β, 9β-H, 14β-H_3. Treatment of **1d** with (*R*)- and (*S*)-MTPA acid in the presence of EDC·HCl and DMAP afforded the (*R*)-MTPA ester (**5**) and (*S*)-MTPA ester (**6**), respectively. The signals due to protons on C-6, 7, and 11 in **5** appeared at higher fields than those of **6** [$\Delta\delta$: positive], while the signals due to protons attached to C-9 and 14 of **5** were observed at lower field as compared to those of **6** [$\Delta\delta$: negative]. Consequently, the absolute configuration at C-8 in **1d** has been elucidated to be *S* and the absolute stereostructure of **1** has been determined as shown.

A 4-epimeric mixture of dihydrocurcumenone was obtained from the transformation products of germacrone by cultured cells, and the stereostructure was deduced on the basis of biogenetic considerations.[5] Oxidation of 4*S*-dihydrocurcumenone (**2**), a colorless oil, $[\alpha]_D^{27}$ −5.1° ($CHCl_3$), $C_{15}H_{24}O_2$, with CrO_3 in pyridine furnished **1**, so that the stereostructure of **2** was clarified except for the 4-position. The modified Mosher's method was also applied to both MTPA esters **7** and **8** prepared from **2**. The signals due to C-1, 2, 3, 5, 6, 9, and 14 in the (*R*)-ester (**7**) appeared in higher fields than those of the (*S*)-ester (**8**), while the signals ascribable to C-15 of **7** were observed

Table 1. ^{13}C-NMR Data for **1d**, **2**, **3**, and **4**

	1d	2	3	4
C-1	29.2	24.2	33.6	23.1
C-2	24.2	25.2	23.4	23.4
C-3	39.4	39.5	43.6	43.8
C-4	110.2	67.8	208.5	208.4
C-5	25.4	24.6	23.8	24.0
C-6	22.2	28.1	29.3	23.7
C-7	43.3	128.3	55.8	54.0
C-8	69.7	202.0	218.8	217.2
C-9	44.2	49.1	50.4	49.2
C-10	18.3	19.9	22.2	18.6
C-11	26.0	147.1	71.6	72.9
C-12	16.2*	23.4	28.5*	28.3*
C-13	20.5*	23.4	25.2*	25.0*
C-14	21.5	19.1	19.9	19.0
C-15	23.7	23.7	30.1	30.1
–O–CH_2–	64.6			

* Assignments may be interchangeable.

Table 2. Inhibitory Effects of Sesquiterpene Constituents from Zedoary on Contractions Induced by High K^+ in Isolated Rat Thoracic Aorta

(Inhibition %)

	High K^+ (50 mM)		
Sample	10 μM	30 μM	100 μM
Control (DMSO)	1.1±3.2	1.4±4.2	0.6±4.6
1	5.4±1.5	12.8±2.7	12.8±2.6
2	2.6±1.2	11.6±3.3	44.9±3.8
3	10.2±3.4	20.9±2.9	39.5±4.2
4	7.6±2.3	20.9±1.2	36.8±0.8
Furanodiene	13.3±1.5	33.9±3.3	60.3±5.9
Germacrone	19.3±3.0	68.4±5.4	94.7±1.8
Curdione	14.8±4.8	23.7±5.4	44.2±7.2
Germacrone-4,5-epoxide	4.8±5.0	17.3±6.8	47.6±6.3
Zederone	10.4±1.8	29.2±3.8	76.7±7.4
13-Hydroxy-germacrone	7.7±3.9	26.1±3.3	74.7±7.2
Dehydrocurdione	6.1±1.2	13.9±2.0	30.3±3.0
Neocurdione	12.5±6.0	30.4±10.4	69.7±12.9
Glechomanolide	9.3±4.8	36.8±9.5	92.0±6.3
Curzerenone	14.9±1.9	38.0±5.5	79.0±8.7
(+)-*ar*-Turmerone	11.0±2.1	37.2±10.3	78.3±6.4
Bisacumol	6.5±2.1	56.5±6.2	75.5±8.1
Bisacurone	−5.4±2.5	0.4±2.0	5.0±1.9
Curcumenol	1.5±0.6	18.5±2.7	54.5±5.9
Isocurcumenol	13.8±3.0	55.3±6.4	88.5±2.5
Aerugidiol	1.1±0.6	1.4±0.8	2.7±0.9
Zedoarondiol	9.2±1.7	11.5±2.9	16.3±3.9
Isozedoarondiol	4.7±2.7	6.0±3.5	8.4±3.9
Zedoalactone B	12.7±3.0	17.1±3.4	21.6±3.8
Alismoxide	11.0±3.1	23.1±2.6	40.0±2.7
7α,11α-Epoxy-5β-hydroxyl-guaiaenone	8.1±0.6	19.0±2.3	43.3±3.8
Zedoarolide A	19.7±3.0	29.1±5.2	42.0±5.8
Zedoarolide B	−1.6±3.3	4.5±3.8	13.5±2.7
β-Eudesmol	29.0±4.1	90.9±3.7	95.0±2.5
β-Dictyopterol	53.1±5.5	93.1±1.4	88.1±3.3
Zedoarofuran	1.3±9.2	11.9±11.3	33.4±13.1
Curcumadione	7.3±1.5	14.3±1.5	27.5±2.1

(N=4—7, mean±S.E.)

in lower fields as compared to that of **8**. This evidence indicated the absolute stereostructure at C-4 in **2** to be *S*.

Curcarabranol A (**3**), a colorless oil, $[\alpha]_D^{26}$ −104.0° ($CHCl_3$), $C_{15}H_{24}O_3$, CD $\Delta\varepsilon$ (MeOH, nm): +1.85 (223), −3.03 (303), EI-MS *m/z*: 252 (M^+), 234 ($M-H_2O$), showed absorption bands due to hydroxyl and carbonyl groups at 3494, 1752, and 1713 cm^{-1} in the IR spectrum. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **3** showed the presence of 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)], an 3-oxobutyl [δ 1.62 (m, 2-H_2), 2.16 (s, 15-H_3), 2.52 (m, 3-H_2)], and a hydroxyisopropyl [δ 1.08, 1.20 (both s, 12, 13-H_3), 4.22 (br s, 11-OH)] together with a *tert*-methyl [δ 1.13 (s, 14-H_3)], two methylenes [δ 1.46, 2.52 (both m, 6-H_2), 2.22, 2.52 (ABq, J=15.0 Hz, 9-H_2)], and a methine [δ 2.37 (dd, J=5.5, 7.0 Hz, 7-H)]. The connectivities of the ^{1}H–^{1}H and the quart. carbons (C-4, 8, 10, 11) in **3** were clarified by ^{1}H–^{1}H COSY and HMBC experiments as shown and its difference NOE experiment of **3** showed NOE correlations between the 14β-methyl proton and the 5β, 7β, and 9β-protons and between the 1α-proton and the 9α-proton. Finally, comparison of the physical data for **3** with those for **1** and **2** led us to depict the structure of **3**. The structure of curcarabranol B (**4**)[9] has been characterized in the same way. The 4,8-dioxo-11-hydroxycarabrane structure of **4** was clarified by ^{1}H–^{1}H COSY and HMBC and its relative stereostructure was determined by difference NOE experiment (NOE correlations: 7α-H & 1α, 9α-H), so that **4** was determined to be the 7-epimer of **3**. To elucidate the total structure of **3** and **4** including the absolute stereostructures, we carried out chemical correlation of **3** and **4** with **1**. That is, acid treatment of **1c** gave **1e** *via* migration of the hydroxyl group from C-8 to C-11 in **1c** and deketalization. Hydroboration of **1e** followed by H_2O_2 oxidation yielded a complex mixture of the triol (**1f**), which was subsequently treated with PCC to give **3** and **4** in a 3 : 1 ratio. On the basis of this evidence, the absolute stereostructures of curcarabranols A (**3**) and B (**4**) were determined as shown.

The vasorelaxant effect of **1**—**4** and principal sesquiterpenes from zedoary were examined using a bioassay to test the inhibitory activity on contractions induced by a high concentration of K^+ in isolated rat aortic strips. As shown in Table 2, many sesquiterpenes except for bisacurone and aerugidiol were found to inhibit the contractions. The vasorelaxant effect of these sesquiterpenes may be related to the traditional medicinal value of zedoary as an emmenagogue and the treatment effect of "Oketsu" syndrome caused by blood stagnation.

References and Notes

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7) All new compounds (**1a—c**, **1e**, **5—8**) were characterized by physicochemical properties and full characteristics will be presented in a full paper.
8) **1d**: colorless oil, $[\alpha]_D^{23}$ +48.1° ($CHCl_3$), $C_{17}H_{30}O_3$. IR (film, cm^{-1}): 3450, 2955, 2872, 1743, 1464, 1219, 1063. ^{1}H-NMR ($CDCl_3$) δ: 0.32 (ddd, J=5.4, 6.1, 7.0, 5-H), 0.40 (dt, J=5.4, 7.0, 1-H), 0.82, 0.88 (both d, J=7.0, 12, 13-H_3), 1.01 (m, 7-H), 1.06, 1.31 (both s, 14, 15-H_3), 1.39 (dd, J=10.0, 11.3, 9α-H), 1.40 (m, 2-H_2), 1.68, 1.71 (both m, 3-H_2), 1.75 (m, 6-H_2), 2.06 (dd, J=4.8, 11.3, 9β-H), 2.11 (dq, J=3.6, 7.0, 11-H), 3.40 (ddd, J=4.8, 5.5, 10.0, 8-H), 3.94 (m, –O–CH_2–CH_2–O–). FAB-MS *m/z*: 305 $(M+Na)^+$.
9) **4**: colorless oil, $[\alpha]_D^{26}$ +77.0° ($CHCl_3$), $C_{15}H_{24}O_3$. CD $\Delta\varepsilon$ (MeOH, nm): +1.30 (208), +0.18 (254), +1.39 (301). IR (film, cm^{-1}): 3494, 2973, 1762, 1709, 1025. ^{1}H-NMR ($CDCl_3$) δ: 0.55 (dt, J=6.9, 7.3, 1-H), 0.72 (m, 5-H), 1.11, 2.15 (both s, 14, 15-H_3), 1.12, 1.17 (both s, 12, 13-H_3), 1.63 (dt, J=7.3, 7.3, 2-H_2), 2.00 (m, 6β-H), 2.07 (m, 7-H), 2.20 (m, 6α-H), 2.46, 2.61 (ABq, J=17.1, 9-H_2), 2.48 (m, 3-H_2), 4.49 (br s, 11-OH). FAB-MS *m/z*: 253 $(M+H)^+$, 235 $(M-H_2O+H)^+$, 251 $(M-H)^-$.