Phytochemistry, Vol. 24, No. 11, pp. 2629–2633, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

CURCUMENONE, CURCUMANOLIDE A AND CURCUMANOLIDE B, THREE SESQUITERPENOIDS FROM *CURCUMA ZEDOARIA**

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(*Received* 7 *March* 1985)

Key Word Index—*Curcuma zedoaria*; Zingiberaceae; zedoary; curcumenone; curcumanolide A; curcumanolide B; cyclopropanosesquiterpene; spirosesquiterpene lactones; (+)-germacrone-4,5-epoxide

Abstract—From the crude drug zedoary (the dried and ground rhizome of *Curcuma zedoaria*) a new cyclopropanosesquiterpene curcumenone and two new spirolactones, curcumanolide A and curcumanolide B have been isolated together with the previously known related sesquiterpenes. The structures and stereochemistries of the new compounds were elucidated by extensive spectral analysis. Young shoots of *C. zedoaria* contain (+)-germacrone-4,5-epoxide, a key intermediate in the biogenesis of germacrone-type sesquiterpenoids. A comparison of the mono- and sesqui-terpenoid constituents of the various parts of the fresh plant with those of zedoary was also made.

INTRODUCTION

The crude drug zedoary, the dried and ground rhizome of *Curcuma zedoaria* Roscoe, has been used medicinally in China [1]. In Japan, it has also been used medicinally, chiefly as an aromatic stomachic. As it contains bioactive principles, the constituents of zedoary have been investigated extensively and it is now recognized to be a rich source of terpenoids. However, these investigations were focused on zedoary produced in Taiwan [2–9] and China [10] and little is known about the drug produced in Japan [11].

In this paper, we report the isolation and structures of three novel sesquiterpenoids from Japanese zedoary and the comparison of the mono- and sesqui-terpenoid constituents of the various parts of the fresh plant with those of zedoary.

RESULTS AND DISCUSSION

Zedoary (dried powder) was extracted with dichloromethane. From the crude extract, three novel sesquiterpenoids named curcumenone (**1**), curcumanolide A (**2**) and curcumanolide B (**3**) were isolated by a combination of column chromatography and preparative HPLC, together with the known components, (+)-germacrone-4,5-epoxide (**4**) [11], germacrone (**5**) [3], furanodienone (**6**) [4], curzerenone (**7**) [5], zederone (**8**) [6], dehydrocurdione (**9**) [7], curcumenol (**10**) [8] and isocurcumenol (**11**) [9].

Curcumenone (**1**), $C_{15}H_{22}O_2$, $[\alpha]_D$ −12.7°, was isolated as a colourless oil. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed the presence of two protons on a cyclopropane ring, two vinylic methyls, two tertiary methyls, four methylene groups, two of which were located at an isolated position, an sp^3 quaternary carbon, a tetrasubstituted double bond and two carbonyl groups. The partial structure **A** for curcumenone (**1**) was deduced by spin decoupling experiments. Irradiation of the quartet at δ1.60 (H-2) caused the double triplet at 0.45 (H-1) and the triplet at 2.47 (H-3) to collapse to a doublet (J = 4.4 Hz) and a singlet, respectively. Reverse irradiation at δ0.45 caused the quartets at 1.60 and 0.67 (H-5) to collapse to triplets (J = 7.3 Hz and J = 4.4 Hz, respectively). Irradiation of the broad singlet at δ2.81 (H-6) collapsed the quartet at 0.67 to a doublet (J = 4.4 Hz) and sharpened the two broad singlets at 1.79 and 2.09 (H-13 and H-12). Reverse irradiation at δ0.67 collapsed the double triplet at 0.45 to a triplet (J = 7.3 Hz) and sharpened the broad singlet at 2.81. The above results coupled with the IR absorption bands at 1718 and 1680 cm^{-1} attributable to conjugated and nonconjugated carbonyl groups, led to structure **1**, having a bicyclo[4.1.0]heptan-3-one skeleton. The relative configuration was confired by the observation of NOEs between 15-Me (δ1.12) and both H-5 and H-2 in the difference spectrum. The structure **1** was in agreement with (+)-curcumenone, $[\alpha]_D$ +11.4° [12] isolated from *Asarum caulescence* Maxim. (Aristolochiaceae). Since the optical rotation of **1** was negative, **1** was the enantiomer of (+)-curcumenone.

Curcumanolide A (**2**), $C_{15}H_{22}O_2$, was isolated as a colourless oil. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) displayed the presence of a secondary methyl, three vinylic methyls, an exocyclic methylene group, three sp^3 methylene groups, one of which was located in an allylic position, two sp^3 methines, a quaternary oxygen-bearing carbon, a tetrasubstituted double bond and a carbonyl group. The partial structufes (**B** and **C**) of **2** were deduced by decoupling experiments. Irradiation of the broad singlet at δ2.47 (H-6) sharpened the two broad singlets at 1.84 and 2.24 (H-13 and H-12). Irradiation of each signal of the exocyclic methylene group at δ4.76 and 4.95 (H-9) sharpened the broad double doublet at 2.82 (H-1) and the

*This work was presented at the 27th Symposium of the Chemistry of Terpenes, Essential Oils and Aromatics of Japan, Nagasaki, October 1983. Symposium papers, p. 61.

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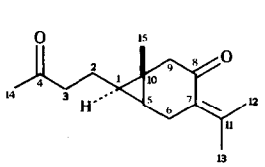

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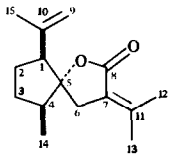

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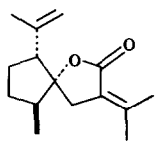

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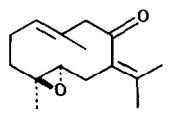

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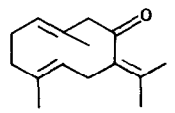

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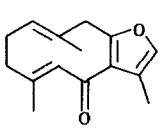

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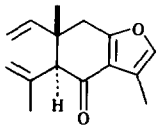

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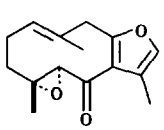

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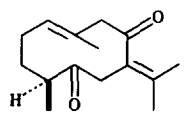

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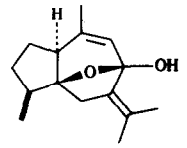

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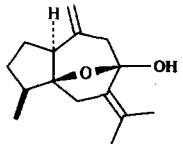

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Table 1. ^{1}H NMR data of compounds 1–3 (400 MHz, $CDCl_3$, TMS as internal standard)*

H	1	2	3
1	0.45 *dt*, *J* = 7.3, 4.4	2.82 *dd* (*br*), *J* = 11.8, 8.8	2.85 *t*, *J* = 7.8
2	1.60 *q*, *J* = 7.3	1.65 *m*	1.62 *m*
		1.80 *m*	2.02 *m*
3	2.47 *t*, *J* = 7.3	1.18 *m*	1.52 *m*
		1.92 *m*	1.84 *m*
4		2.32 *m*	1.86 *m*
5	0.67 *q*, *J* = 4.4		
6	2.81 *m*	2.47 *s* (*br*)	2.49 *d* (*br*), *J* = 16.6
			2.87 *d* (*br*), *J* = 16.6
9	2.52 *d*, *J* = 15.6	4.76 *s* (*br*)	4.76 *s* (*br*)
	2.55 *d*, *J* = 15.6	4.95 *s* (*br*)	4.89 *s* (*br*)
12	2.09 *s* (*br*)	2.24 *s* (*br*)	2.25 *s* (*br*)
13	1.79 *s* (*br*)	1.84 *s* (*br*)	1.84 *s* (*br*)
14	2.13 *s*	0.87 *d*, *J* = 6.8	0.97 *d*, *J* = 6.4
15	1.12 *s*	1.73 *s* (*br*)	1.67 *s* (*br*)

*All assignments were confirmed by the double resonance experiments.

broad singlet at 1.73 (H-15), respectively. Reverse irradiation at δ2.82 sharpened the broad singlet at 1.73 and affected the two multiplets at 1.65 and 1.80 (H-2). Irradiation of the multiplet at δ2.32 (H-4) collapsed the doublet at 0.87 (H-14) to a singlet and affected the two multiplets at 1.18 and 1.92 (H-3). Further decoupling experiments indicated that the two methylene groups (H-2 and H-3) were vicinal. These results indicated that **2** possessed the partial structures **B** and **C**. The spiro structure was assigned based on the presence of a quaternary oxygen-bearing carbon by ^{13}C NMR. The ^{1}H and ^{13}C NMR spectral data coupled with the IR (1738 cm^{-1}) and UV (235 nm) absorption ascribed to the carbonyl group led to the structure **2** for curcumanolide A. Since NOE difference spectra of **2** revealed the proximity of the 14-Me group to H-6, and of H-1 to H-4 (Fig. 1), the relative stereochemistry of **2** was as shown in the formula.

Curcumanolide B (**3**), $C_{15}H_{22}O_2$, a colourless oil was thought to be a stereoisomer of **2**, since its ^{13}C NMR (Table 2) was very similar to that of **2**. The proton signals were assigned by spin decoupling experiments and the results were shown in Table 1. In the difference spectrum, NOEs were observed between the 14-Me and H-6, and between 14-Me and H-1 (Fig. 1) indicating that **3** was the C-1 epimer of **2**. Thus, the structure of curcumanolide B

Table 2. ^{13}C NMR data of compounds 1–3 (22.6 MHz for 1, 100 MHz for 2 and 3, $CDCl_3$, TMS as internal standard)

C	1	2	3
1	24.2 *d*	52.3 *d*	56.0 *d*
2	23.2 *t*	26.5** *t*	33.8** *t*
3	43.9 *t*	23.3** *t*	30.8** *t*
4	208.8 *s*	42.8 *d*	45.2 *d*
5	24.2 *d*	89.6 *s*	91.6 *s*
6	28.0 *t*	27.6‡ *t*	27.4‡ *t*
7	128.2 *s*	120.9 *s*	120.8 *s*
8	201.6 *s*	169.9 *s*	169.8 *s*
9	48.9 *t*	112.7 *t*	114.0 *t*
10	20.1 *s*	149.1 *s*	148.9 *s*
11	147.4 *s*	143.8 *s*	145.3 *s*
12	23.4 *q*	24.3†*q*	24.4†*q*
13	23.4 *q*	23.9†*q*	22.0†*q*
14	30.0 *q*	13.1 *q*	13.1 *q*
15	19.0 *q*	19.9 *q*	19.9 *q*

*† Values with the same superscript in the same column are interchangeable.

‡ Signals assigned by selective decoupling.

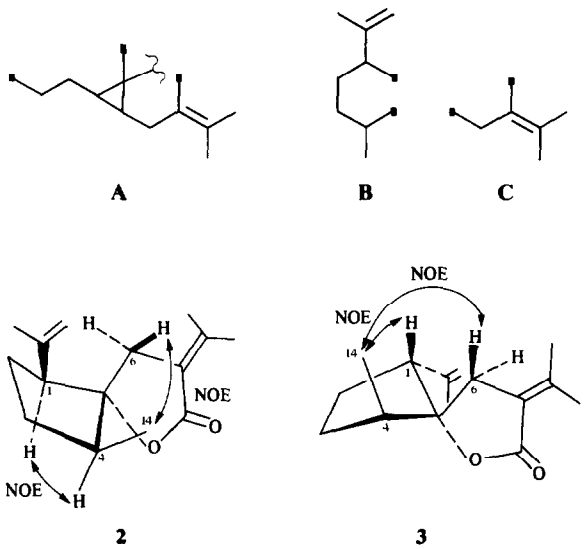

Fig. 1.

was established as **3**. Finally, compounds **2** and **3** were identified with the products of acid-catalysed transformation of dehydrocurdione (**9**) [13].

(−)-Germacrone-4,5-epoxide (enantiomer of **4**) a possible biogenetic precursor of (+)-curcumenone (enantiomer of **1**) has been isolated from *A. caulescence* [14]. The occurrence of (−)-curcumenone (**1**) in *C. zedoaria* prompted us to search for the (+)-form of germacrone-4,5-epoxide (**4**) in the same plant. (±)-Germacrone-4,5-epoxide prepared by the oxidation of germacrone (**5**) with *m*-chloroperbenzoic acid [14] showed the same retention time and mass spectrum as those of **1** on GC-MS. On preparative GC, (±)-**4** at 240° (inject. temp.) underwent thermal rearrangement to yield (±)-curcumenone (**1**). These facts mean that it is difficult to distinguish between **1** and **4** by GC analysis. Investigations based on these findings of various parts of the fresh plant showed that (+)-germacrone-4,5-epoxide (**4**) was present as the major component in young shoots. As expected, the epoxide (**4**) thus obtained showed the reverse (positive) CD spectrum to that of (−)-**4** isolated from *A. caulescence*. More recently, Yoshihara *et al.* [11] reported the isolation of (+)-**4** from *C. zedoaria* and determined its absolute configuration. From a biogenetic point of view, the correlation of absolute configuration between dehydrocurdione (**9**) [13] and **4** suggests that **9** may be formed by rearrangement of the epoxide ring of **4**. We have realized the biomimetic transformation of **9** into curcumenol (**10**) and isocurcumenol (**11**) [13]. On the basis of these results, possible biogenetic pathways for the formation of the sesquiterpenoids found in *C. zedoaria* are shown in Fig. 2.

Little attention has been paid to the chemical constituents of fresh rhizome, young shoot, leaf and root of *C. zedoaria*. Each organ was extracted with dichloromethane and the crude extracts directly analysed by GC-MS equipped with a computer. The mass spectra obtained were identified by direct comparison with those of authentic samples. Table 3 shows the chemical constituents isolated or detected. As seen in Table 3, all of the parts, except root, contained **9** as the major component. In dried powder of rhizome, the monoterpenoid content is low and the presence of the spirolactones (**2** and **3**) and curzerenone (**7**) is characteristic. It is noteworthy that young shoot contains **4** and **5**, key intermediates in the biosynthesis of germacrone-type sesquiterpenoids in *C. zedoaria*, as the major components. In leaf, elemenes and sesquiterpenes possessing a furan ring have not been detected. Furthermore, the root is chemically different from the other parts, since it produces bornyl acetate as the major component and neither germacrone-type nor furanosesquiterpenes have been found. Thus, each part of the plant is characterized by its chemical constituents.

EXPERIMENTAL

Mps: uncorr; ^1H NMR: 400 MHz, TMS–$CDCl_3$; ^{13}C NMR: 22.6 or 100 MHz, TMS–$CDCl_3$; $[\alpha]_D$ and CD: $CHCl_3$ or MeOH; UV 95% EtOH; IR: $CHCl_3$ or liquid film. TLC: precoated silica gel (0.25 mm) F_{254}, *n*-hexane–EtOAc (4:1) or CH_2Cl_2. Spots were detected by I_2 vapour, UV light (254 nm) or spraying with Ehrlich's reagent. Prep. GC: SE-30 1%, glass column (2 m × 2 mm), temp. programme 100–230° at 5°/min, inject. temp. 240°, He 30 ml/min. GC-MS: 70 eV, SE-30 1%, glass column (2 m × 2 mm), temp. programme 50–270° at 5°/min. HPLC: μ Porasil, *n*-hexane–EtOAc (97:3) as solvents.

Plant materials. Zedoary (dried powder) and fresh rhizomes of *Curcuma zedoaria* were purchased from Yakushima Island and samples were deposited in the Herbarium of the Institute of Pharmacognosy, Tokushima Bunri University. Fresh rhizomes were hydroponically cultured at 37° under dark condition for 3 weeks and young shoots (58 g) and roots (5 g) were collected. Fresh rhizomes were also planted in the botanical garden of Tokushima Bunri University and leaf material was collected in July.

Extraction and isolation of sesquiterpenoids from zedoary. Zedoary (16 kg) was extracted with CH_2Cl_2 for 3 days and the CH_2Cl_2 soln was concd *in vacuo* to give a dark brown oil (500 g) 5.0 g of which was directly chromatographed in silica gel (100 g) using an *n*-hexane–EtOAc gradient (*n*-hexane, 400 ml); 99:1 200 ml; 98:2, 600 ml; 97:3, 750 ml; 95:5, 1500 ml). The eluant was

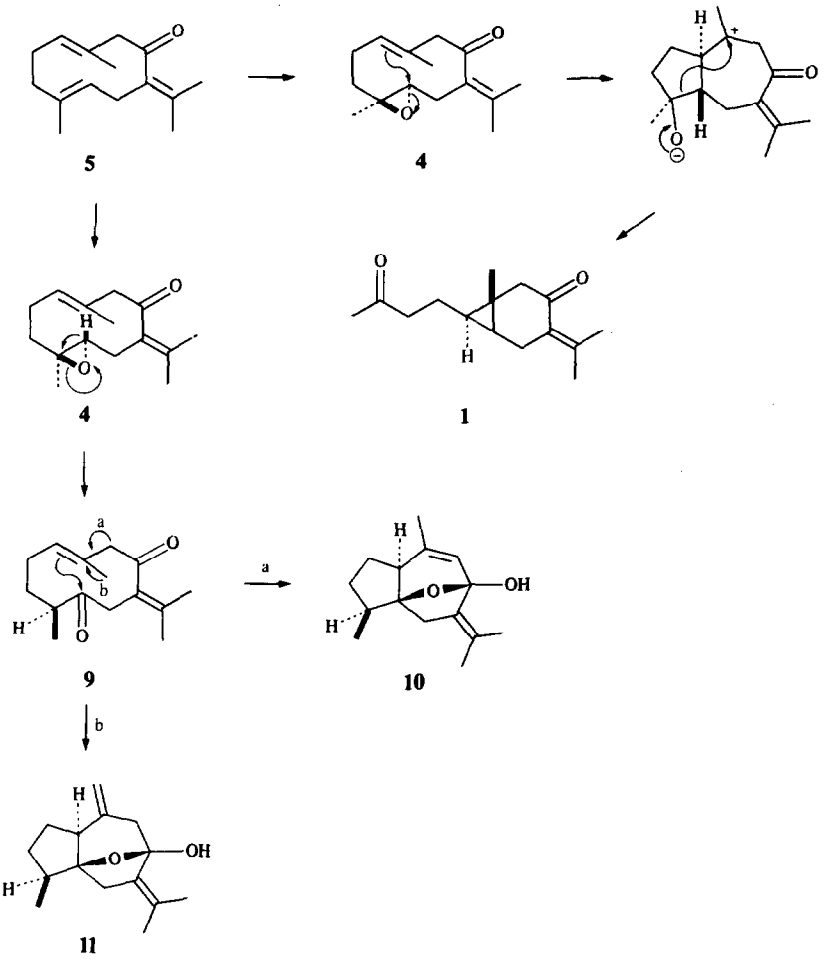

Fig. 2. Possible biogenetic pathways for the formation of the sesquiterpenoids found in *C. zedoaria*.

collected as 50 ml fractions. Fraction 14 was concd and the residue was rechromatographed on Sephadex LH-20 using $CHCl_3$–MeOH (1:1) to give germacrone (**5**) (22 mg), mp 50–51° [3]. The residue obtained from fractions 15–17 was rechromatographed on silica gel using *n*-hexane–EtOAc (2:1) to afford curzerenone (**7**, 445 mg), $[\alpha]_D$ 0° [5] as a colourless oil. Fractions 19–22 were combined and treated in the same manner as described above to give furanodienone (**6**) (127 mg), mp 89–91° (lit. 89.5–90.5° [4]). Fractions 23–25 contained two spirolactones (124 mg) which were purified by prep. HPLC to afford curcumanolide A (**2**, 23 mg) and curcumanolide B (**3**, 14 mg). *Compound* **2**. Colourless oil; $[\alpha]_D$ −33.0° (*c* 1.2; $CHCl_3$); IR $\nu_{max}^{liq.}$ cm^{-1}: 1738, 1666; UV λ_{max} nm (log ε): 235 (4.15); High resolution MS *m/z*: 234.1617 (calc. for $C_{15}H_{22}O_2$: 234.1618); MS *m/z* (rel. int.): 234 $[M]^+$ (7), 219 (7), 191 (20), 178 (56), 165 (78), 164 (100), 152 (72), 121 (28), 109 (26), 96 (30), 69 (45), 68 (44), 67 (45). *Compound* **3**. colourless oil; $[\alpha]_D$ +24.2° (*c* 1.4; $CHCl_3$); IR $\nu_{max}^{liq.}$ cm^{-1}: 1740, 1644; UV λ_{max} nm (log ε): 235 (4.15); High resolution MS *m/z*: 234.1612 (calc. for $C_{15}H_{22}O_2$: 234.1618); MS *m/z* (rel. int.): 234 (36), 219 (7), 191 (18), 178 (57), 165 (85), 164 (100), 152 (78), 121 (22), 109 (24), 96 (30), 69 (43), 68 (44), 67 (44). Each residue obtained from fractions 28–32 and 34–40 was rechromatographed on silica gel using *n*-hexane–CH_2Cl_2 (1:2) to give dehydrocurdione (**9**, 502 mg) $[\alpha]_D$ +65° (*c*, 1.0; $CHCl_3$) (lit. +67.9° [7]) and isocurcumenol (**11**, 61 mg), mp 145–146°; $[\alpha]_D$ +40° (*c* 0.5) (lit. 139–141°; $[\alpha]_D$ +34° [9]), respectively. The residue obtained from combined fractions 44–46 was rechromatographed on silica gel using CH_2Cl_2 to give zederone (**8**, 180 mg), mp 149–150°; $[\alpha]_D$ +272° (*c* 1.0; $CHCl_3$) (lit. mp 153.5–154°; $[\alpha]_D$ +265.8° [6]). Fractions 54–57 were combined and the residue was rechromatographed on silica gel using CH_2Cl_2 to afford curcumenol (**10**, 104 mg), mp 119–120°; $[\alpha]_D$ +385° (*c* 1.0; $CHCl_3$) (lit. mp 118.5–119.5°; $[\alpha]_D$ +397° [8]). Fractions 61–69 gave an oil which was rechromatographed on silica gel using CH_2Cl_2–$CHCl_3$ (1:1) to afford curcumenone (**1**, 136 mg) as a colourless oil. $[\alpha]_D$ −12.7° (*c*, 1.1; MeOH) (lit. +11.4° [Endo, J. (1983) personal communication]); IR $\nu_{max}^{liq.}$ cm^{-1}: 1718, 1680, 1600; UV λ_{max} nm (log ε): 248 (3.92); High resolution MS: *m/z* 234.1612 (calcd for $C_{15}H_{22}O_2$: 234.1618); MS *m/z* (rel. int.): 234 (29), 219 (13), 191 (17), 176 (60), 167 (32), 163 (31), 161 (32), 149 (40), 121 (33), 107 (30), 68 (100), 67 (48), 43 (65).

Isolation of (+)-germacrone-4,5-epoxide (**4**) *from young shoot.* Fresh young shoots (58 g) were sliced and extracted with CH_2Cl_2 for 3 days. The crude extract (130 mg) was directly chromatographed on silica gel (5 g) using CH_2Cl_2 and 20 ml fractions were collected. Fractions 8–10 were combined and the residue after evaporation of the solvent was rechromatographed on Sephadex LH 20 using $CHCl_3$–MeOH (1:1) to give (+)-germacrone-4,5-epoxide (**4**, 4.4 mg), mp 62–64°; $[\alpha]_D$ +238° (*c* 0.22; MeOH); CD $[\theta]_{308\,nm}$ +9000 (*c* 0.002; MeOH) (lit. mp 59–60°; $[\alpha]_D$ +399° in $CHCl_3$): $[\theta]_{308\,nm}$ +15000 [11]). The ^1H NMR spectral data of **4** were identical to those of synthetic (±)-germacrone-4,5-epoxide

Table 3. Chemical constituents of *Curcuma zedoaria*

	Japanese						
	Zedoary	Fresh rhizome	Young shoot	Leaf	Root	Taiwan zedoary [2–9]	Chinese zedoary [10]
α-Pinene				+			
Camphene		+		+			
β-Pinene		+	+	+			
1,8-Cineole	+	++	++	+++	+		
Linalool				+	+		
Camphor	+	+	+	++			
α-Terpinene				+			
Borneol					+		
Bornyl acetate					+++		
δ-Elemene	+	+	+				
β-Elemene	+	+	+		++		
β-Caryophyllene		+	+	+	++		
α-Humulene					+		
trans-β-Farnesene			++				
γ-Elemene		+	+		+		
Furanogermenone							+
Curcumenone (1)	++	++		++*			
Curcumanolide A (2)	++						
Curcumanolide B (3)	+						
Germacrone-4,5-epoxide (4)		+	+++	++*			
Germacrone (5)	+	+	++	+		+	
Furanodienone (6)	+	++	++			+	
Curzerenone (7)	++					+	
Zedcrone (8)	+	+	+			+	
Dehydrocurdione (9)	+++	+++	+++	+++		+	
Curcumenol (10)	++	++	+			+	
Isocurcumenol (11)	+	+	+			+	

*Compound 1 and/or germacrone-4,5-epoxide (4).

(4) [14]. The mass spectrum of 4 obtained from GC-MS was the same as that of curcumenone (1) formed from 4 by pyrolysis in GC.

Thermal rearrangement of (±)-germacrone-4,5-epoxide (4). (±)-Germacrone-4,5-epoxide (4) was subjected to prep. GC (conditions: see previous paragraph) and the component corresponding to *R*, 12.4 min was collected. The spectral data of the colourless oil thus obtained, except optical rotation, were identical to those of curcumenone (1).

Identification of mono- and sesqui-terpenoids in fresh rhizome, young shoot, leaf and root. Each part of *C. zedoaria*, was extracted with CH_2Cl_2. The extracts after removal of the solvent were analysed directly by TLC, GC and GC-MS equipped with a computer. The components were identified by comparison of their mass spectra with those of authentic samples.

Acknowledgements—We wish to thank to Dr. I. Horibe, Shionogi and Co. Ltd., Osaka, Japan and Dr. J. Endo, Science University of Tokyo for their valuable discussion on the structure of 1. We are also indebted to Prof. T. Takahashi, University of Tokyo, for the measurements of high resolution mass spectra. The present work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfate for Cancer Research (Y.A.).

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