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GUAIANE SESQUITERPENE LACTONES FROM *CURCUMA AERUGINOSA*

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Key Word Index— *Curcuma aeruginosa* (= *C.zedoaria*); rhizomes; Zingiberaceae; guaiane-type sesquiterpene lactones; zedoalactone A; zedoalactone B.

Abstract—Two guaiane derivatives were isolated from the rhizomes of *Curcuma aeruginosa* (= *C. zedoaria*). Their structures [(1*S**, 4*S**, 5*S**, 8*R**, 10*S**)-4,10-dihydroxyguai-7(11)-en-12,8-olide (zedoalactone A) and (1*R**, 4*S**, 5*R**, 10*R**)-1,4,10-trihydroxyguai-7(11),8-dien-12, 8-olide (zedoalactone B)] were established by ^{1}H and ^{13}C NMR spectroscopic studies and by comparison with closely related compounds. In addition, the already known guaianolide zedoarondiol was isolated from the same source.

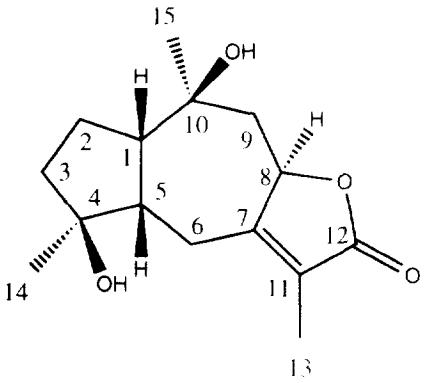
INTRODUCTION

Curcuma spp. (Zingiberaceae) is widespread in east and southern Asia, and has long been used as a gastrointestinal remedy and a spice. In Japan, the rhizome of *C.aeruginosa* RoxB. (= *C. zedoaria* Rosc.) [1] is called 'Gajutsu', and is used as an oriental traditional medicine. So far, many kinds of sesquiterpenoids have been isolated from *Curcuma* spp [2–7]. Recently, we have isolated highly oxygenated guaiane type sesquiterpene lactones from *n*-butanol extract of the title plant. We report herein, the isolation and characterization of these sesquiterpenoids.

RESULTS AND DISCUSSION

The aqueous methanol extract from dried and powdered rhizomes was partitioned with *n*-hexane, ethyl acetate and *n*-butanol successively. The crude *n*-butanol extract was chromatographed on DIAION HP20, ODS silica gel or reversed phase preparative HPLC and yielded two new guaiane type sesquiterpene lactones, zedoalactone A (**1**) and zedoalactone B (**2**), together with the previously known zedoarondiol (**3**) [6,7].

Compound **1** was obtained as colourless powder. The EI mass spectrum of **1** did not show the molecular ion. The highest peak in **1** appeared at *m/z* 248 as a dehydrated ion $[M - H_2O]^+$HR mass spectrometry (MS), found 248.1407, $C_{15}H_{20}O_3$, which suggested a molecular formula $C_{15}H_{22}O_4$. The IR spectral data showed a hydroxyl group (3390 cm^{-1}) and α,β-unsaturated γ-lactone (1730 cm^{-1}). The ^{1}H NMR (pyridine-d_5) spectrum (Table 1) indicated the presence of three tertiary methyls (δ1.35*s*, 1.54*s*, 1.81*d*), four sets of methylene protons, two methine protons and a carbinyl proton (δ5.09 *ddd*). The ^{13}C NMR (pyridine-d_5) spectrum (Table 2) contained 15 carbons; three tertiary methyls (δ8.1, 25.4, 31.5), four methylenes (δ25.2, 25.3, 37.8, 38.3) and three methines (δ51.4, 52.8, 81.2). It was similar to those of some guaianolides [6–8]. The ^{13}C NMR spectrum of **1** showed the parallel chemical shift pattern to isozedoarondiol **4** [7] at C-1 to C-5. However, it showed the remarkable downfield shifts at C-7 (δ162.4) and C-12 (δ175.5), upfield shifts at C-8 (δ81.2), C-9 (δ37.8), C-11 (δ121.9) and C-13 (δ8.1) compared with those of **4**. These spectral data indicated that **1** possessed a hydroxygenated guaiane skeleton closely related to **4**, having an α,β-unsaturated γ-lactone moiety on the B-ring system. The ^{1}H–^{1}H COSY spectrum [9] of **1** revealed the partial structure -CH (1)-CH_2 (2)-CH_2 (3)-, CH (5)-CH_2 (6)- and CH (8)-CH_2 (9)-. The ^{13}C–^{1}H COSY [10] and the COLOC [11] spectrum revealed the relationship of the tertiary or the quaternary carbons with the protons of **1**. H-13 were correlated with three quaternary carbons (C-7, C-11, C-12), and also both H-6 and H-8 with (C-7, C-8, C-11) on the γ-lactone



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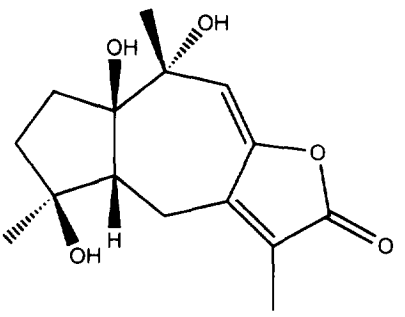

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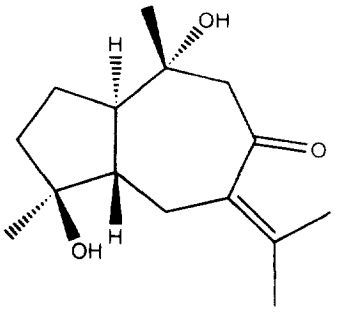

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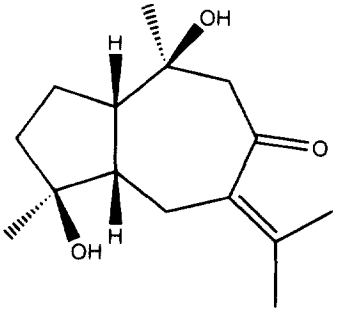

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ring. The tertiary methyl protons H-14 were correlated with three carbons (C-3, C-4, C-5) and also H-15 with three carbons (C-1, C-9, C-10). The above spectral evidence suggested that **1** was 4,10-dihydroxyguai-7 (11)-en-12, 8-olide. The sterochemistry of **1** was confirmed by the coupling constants, NOE enhancements [12] as well as pyridine-induced solvent shifts [13] in the ^{1}H NMR. The signals at H-1 (δ3.25) and H-5 (δ2.49) of the guaiane framework showed a coupling (J = 7.0 Hz) and a NOE enhancement, which indicated that the A/B-ring linkage was the *cis*-configuration. The coupling constants of H-5 in the axial orientation showed the ABX type vicinal coupling with C-6 methylene protons, H-6αax (δ1.96, J = 13.4 Hz) and H-6β eq (δ2.77, J = 3.7 Hz). H-8ax (δ5.09) showed the ABX type vicinal coupling with H-9 αax (δ2.45, J = 7.0 Hz) and H-9β eq (δ2.20, J = 3.7 Hz) and also showed the NOE enhancement with H-6α and H-9α. Thus, H-8 had the α-orientation. Furthermore, remarkable pyridine-induced solvent shifts were observed for H-1 (δ pyridine–δ methanol = + 0.6 ppm), H-9 β (+ 0.3 ppm), H-5 (+ 0.5 ppm) and H-3 β (+ 0.3 ppm). These deshielding effects suggested that these protons were situated in the vicinal position of hydroxyl groups and disposed towards the same side of the molecule. Therefore, it was evident that the orientation of both tertiary hydroxyl groups attached to C-4 and C-10 were β. From the spectral data the relative structure of **1** was identified as (1S^*, 4S^*, 8R^*, 10S^*)-4,10-dihydroxyguai-7(11)-en-12,8-olide. The conformation of **1** had the twist-chair form, depicted as in Fig.1, from the results of the coupling constants of B-ring protons and the NOE enhancements.

Compound **2** was obtained as a colourless oil. The EI mass spectrum of **2** showed a dehydrated ion peak $[M - H_2O]^+$ at m/z 262 (HR-MS, found: 262.1195, $C_{15}H_{18}O_4$, which suggested a molecular formula $C_{15}H_{20}O_5$. The IR spectral data showed the partial structure of a hydroxyl group (3400 cm^{-1}) and α,β-unsaturated γ-lactone (1740 cm^{-1}). The UV spectrum (273 nm) of **2** showed a 50 nm bathchromic shift compared with that of **1**, indicating the presense of another unsaturated bond conjugated with the lactone ring. The ^{1}H NMR pyridine-d_5) spectrum showed three tertiary methyls (δ1.71 *d*, 1.75 *brs*, 1.90 *s*), three sets of methylene protons, a methine proton (δ3.35 *dd*) and an an olefinic proton (δ6.09 *s*). The ^{13}C NMR spectrum contained 15 carbons, three tertiary methyls (δ8.4, 23.7, 26.1), three methylenes (δ22.0, 35.7, 41.5) and two methines (δ50.3, 118.8). Compared with those of **1**, there were some different points at B-ring carbons. Compound **2** showed the

Table 1. ^{1}H NMR spectral data of compounds **1–3*** [in pyridine-d_5, 500 MHz (mult.: J/Hz)]

H	1	2	3
1	3.25 *ddd* (7.0, 7.0, 11.6)	—	2.35 *ddd* (4.0, 8.0, 11.5)
2α	1.61 *m*	2.06 *ddd* (8.0, 11.5, 13.1)	1.98 *m*
2β	1.98 *m*	3.10 *ddd* (2.0, 9.0, 13.1)	1.98 *m*
3α	1.90 *m*	2.15 *ddd* (2.0, 8.0, 11.5)	1.87 *m*
3β	2.06 *m*	2.41 *ddd* (9.0, 11.5,11.5)	1.97 *m*
5	2.49 *ddd* (7.0, 3.7, 13.4)	3.35 *dd* (3.0, 12.8)	1.80 *brt* (11.5)
6α	1.96 *dd* (13.4, 12.5)	3.21 *ddd* (1.5, 12.8, 17.4)	2.12 *dd* (11.5, 14.5)
6β	2.77 *dd* (3.7, 12.5)	3.08 *ddd* (1.5, 3.0, 17.4)	3.11 *brd* (14.5)
8	5.09 *ddd* (1.8, 3.0, 7.3)	—	—
9α	2.45 *dd* (7.3, 15.5)	6.09 *s*	3.35 *d* (12.5)
9β	2.20 *dd* (3.7, 15.5)	—	3.05 *d* (12.5)
12Me	—	—	1.99 *s*
13Me	1.81 *d* (1.8)	1.71 *d* (1.5)	1.73 *s*
14Me	1.54 *s*	1.75 *brs*	1.35 *s*
15Me	1.35 *s*	1.90 *s*	1.43 *s*
1-OH	—	7.12 *s*	—
4-OH	5.05 *brs*	6.22 *s*	6.04 *brs*
10-OH	5.92 *brs*	6.02 *s*	5.88 *brs*

*Assignments from C–H correlation experiments.

Table 2. ^{13}C NMR spectral data of compounds **1–3*** (in pyridine-d_5, 125 MHz)

C	1	2	3
1	52.8	75.1	55.7
2	25.3	35.7	21.7
3	38.3	41.5	39.5
4	80.6	79.5	79.8
5	51.4	50.3	51.6
6	25.2	22.0	28.3
7	162.4	151.2	134.6
8	81.2	148.5	203.3
9	37.8	118.8	59.7
10	72.4	82.7	72.6
11	121.9	125.8	142.2
12	175.5	170.2	22.1
13	8.1	8.4	22.8
14	25.2	23.7	22.4
15	31.5	26.1	20.3

*Assignments from C–H correlation experiments.

downfield shift at C-1 (δ75.1) suggesting a carbinyl carbon. An unsaturated bond between C-8 (δ148.5) and C-9 (δ118.8) conjucated with a double bond C-7 (δ151.2) and C-12 (δ170.2) in the α,β-unsaturated γ-lactone moiety. The ^{1}H–^{1}H COSY spectrum of **2** revealed the partial structure -CH_2(2)-CH_2(3)- and CH(5)-CH_2(6)-. The ^{13}C–^{1}H COSY and the COLOC spectra indicated that three tertiary methyl groups were attached the same positions as in **1**. From the above spectral evidence, compound **2** was confirmed as 1, 4,10-trihydroxyguai-7(11),8-dien-12,8-olide. The NOE enhancements of the C-1 hydroxyl proton with the H-2β, H-5 and H-15 methyl protons indicated that the C-1 hydroxyl group

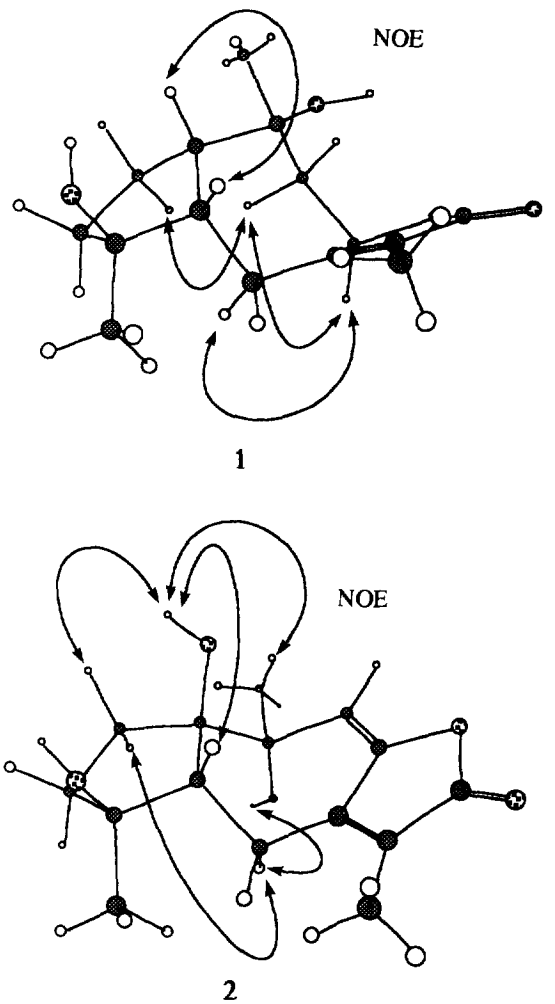

Fig. 1. NOE enhancements of **1** and **2**.

was in the β-orientation and the A/B-ring linkage had the *cis*-configuration. From the NOE enhancements between the C-4 hydroxyl proton with H-3 β and H-5, it was evident that the C-4 hydroxyl group was also in the β-orientation. Furthermore, the NOE enhancements of the C-10 hydroxyl proton with H-2 α and H-6 α showed the C-10 hydroxyl group was in the α-orientation. These conclusions were also supported by the remarkable pyridine-induced solvent shifts observed in H-2β (δ pyridine–δ methanol = + 0.3 ppm), H-3 β (+ 0.4 ppm) and H-5 (+ 0.6 ppm). Thus, the relative structure of **2** was reveiled as (1R^*,4S^*, 5R^*, 10R^*)-1, 4,10-trihydroxyguai-7(11),8 -dien-12, 8-olide. The coupling constants of the B-ring protons of **2** were the same as those of **1** and also supported the NOE. The H-5 angular proton showed ABX type vicinal coupling with the C-6 methylene protons as H-6αax (δ3.21, J = 12.8 Hz) and H-6β eq (δ3.08, J = 3.0 Hz). From the spectral data it was deduced that the conformation of **2** was the boat form as depicted in Fig.1.

Structural confirmation of **3** was obtained by MS, optical properties and ^{1}H and ^{13}C NMR spectroscopy, in almost full agreement with the corresponding data for zedoarondiol [7]. In our 2D NMR experiment, the complete assignment of **3** was established. The C–H long range correlation of C-3 with H-14 methyl protons (δ1.33*s*) in the COLOC spectra and the vicinal coupling with C-6 methylene protons, H-6α δ1.97 and H-6β 2.82) with the H-5 angular methine proton in the ^{1}H–^{1}H COSY spectra revealed that the ^{13}C NMR assignments for C-3 (δ28.5) and C-6 (δ39.7) of **3** cited in the literature [7] must be revised as C-3 (δ39.5) and C-6 (δ28.3).

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra: CD_3OD or pyridine-d_5 with TMS as int. standard. NOESY experiments were made with a mixing time of 0.85 sec. EI-MS: 70 eV, IR: KBr, UV: CH_3OH. HPLC was performed with a TSK gel ODS-120T column (300 × 22 mm i.d. Toso) packed with 10 μm ODS. TLC was conducted on precoated Kieselgel. Spots on TLC were detected by their absorption under UV light. NMR coupling constants (J) are given in Hz.

Materials. The rhizomes of *C. aeruginosa* used in this experiment were cultivated in Yakushima island, Japan, in June 1992, and voucher specimens are deposited in the Herbarium of the Tokyo Metropolitan Research Laboratory of Public Health, Tokyo, Japan.

Extraction and isolation of **1**–**3**. Dried and powdered rhizomes (1.4 kg) of *C. aeruginosa* were extracted with MeOH (3 × 3 l) at room temp. for 5 days to give the extract (84 g). The MeOH extract was suspended in H_2O (1.5 l) and extracted (× 3) with *n*-hexane (1.5 l), EtOAc (1.5 l) and then with *n*-BuOH (1.5 l). The *n*-BuOH soluble phase was concd to give crude extract (17 g). The extract was suspended in H_2O (100 ml) and applied to a DIAION HP20 column (700 g) which was conditioned with H_2O. Using H_2O–MeOH mixts of increasing MeOH concn (0, 30, 60, 100%) gave four frs, which were monitored by TLC and HPLC. Frs eluted with 60% MeOH were combined and processed by ODS column or reversed phase HPLC using MeCN–H_2O solvent system to give **1** (130 mg), **2** (90 mg) and **3** (180 mg).

Zedoalactone A (**1**). Powder, $[\alpha]_D$ – 34.3° (MeOH; *c* 0.4). EIMS *m/z* (rel. int.):[M]$^+$absent, 248 [M – H_2O]$^+$ (17), 230 [M – 2H_2O]$^+$ (33), 226 [M – 3H_2O]$^+$ (100) and 215 [M – 2H_2O – CH_3]$^+$ (23), 201 (37), 187(20). (HR-MS, found: [M – H_2O]$^+$, 248.1407. $C_{15}H_{20}O_3$ requires [M – H_2O]$^+$, 248.1413). IR ν_{max}^{KBr} cm^{-1}: 3390*br*, 2970, 1730 and 1680. UV λ_{max}^{MeoH} nm: 223 (log ε 4.00). ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Zedoalactone B (**2**). Oil, $[\alpha]_D$ + 177.7° (MeOH; *c* 0.4). EIMS *m/z* (rel. int): [M]$^+$ absent, 262 [M – H_2O]$^+$ (13), 244 [M – 2H_2O]$^+$ 33, 226 [M – 3H_2O]$^+$ (100) and 211 [M – 3H_2O – CH_3]$^+$ (66). HR-MS, found: [M – H_2O]$^+$, 262.1195. $C_{15}H_{18}O_4$ requires [M – H_2O]$^+$, 262.1205. IR ν_{max}^{KBr} cm^{-1}: 3400, 2970, 2940, 2880, 1740, 1660 and 1630. UV λ_{max}^{MeOH} nm: 273 (log ε 4.33). ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Zedoarondiol (**3**). Oil, $[\alpha]_D$ – 39.5° (MeOH; *c* 0.4). EIMS *m/z*: 252 [M]$^+$ (5), 234 [M – H_2O]$^+$ (43), 216 [M – 2H_2O]$^+$ (50), 201(25), 191(45), 173(62), 145(70), 131(50), 119(42), 104(100). (HR-MS, found: [M]$^+$, 252.1715. $C_{15}H_{24}O_3$ requires [M]$^+$, 252.17226). IR ν_{max}^{KBr} cm^{-1}: 3390, 2970, 1665 and 1605. UV λ_{max}^{MeOH} nm: 254 (log ε 3.75). ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

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