The Absolute Structure and Synthesis of Wenjine Isolated from *Curcuma wenyujin*

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A novel highly oxygenated sesquiterpenoid named wenjine (1) was isolated from *Curcuma wenyujin*. The relative structure was established on the basis of spectroscopic data and X-ray analysis. The absolute structure of 1 was determined by photooxygenation of (+)-(1*S*,10*S*),(4*S*,5*S*)-germacrone-1(10),4-diepoide (2).

Keywords wenjine; *Curcuma wenyujin*; Zingiberaceae; sesquiterpene; germacrone diepoide; photooxidation; hemiacetal dioxalane

In our continuing study of bioactive constituents of *Curcuma wenyujin* (Zingiberaceae), seven sesquiterpenes, curcuml,²⁾ curdione,^{3,4a)} curcumalactone,⁴⁾ (1*R*,10*R*)-(–)-1,10-dihydrocurione,⁵⁾ neocurdione,⁶⁾ (1*S*,10*S*),(4*S*,5*S*)-germacrone-1(10),4-diepoide (2),⁷⁾ and (4*S*,5*S*)-(+)germacrone-4,5-epoxide (3),^{7,8)} have been isolated from the essential oil⁹⁾ of the rhizome. In this paper, we wish to describe the absolute structure of a new sesquiterpene dioxalane hemiacetal named wenjine (1) and chemical correlations of the monoepoxide (3) and diepoide (2).^{7,8)}

Air-dried chipped rhizomes of *C. wenyujin* were percolated with ether at 5°C for a week. Evaporation of the solvent afforded an essential oil. This oil was submitted to silica gel chromatography with petroleum ether and ether solvent system. The fractions eluted with petroleum ether–ether (5:4) afforded (1*S*,10*S*),(4*S*,5*S*)-germacrone-1(10),4-diepoide (2). The 5:6 fractions were collected and evaporated to dryness to yield an oily substance. This oil was submitted to silica gel chromatography (petroleum ether–ether; 5:1) to yield a crystalline material. Recrystallization from methanol–chloroform (1:1) afforded wenjine (1) as colorless prisms.

Wenjine (1), mp 230–231°C, $[\alpha]_D^{25} +54.3^\circ$ ($c=0.37$, dimethylformamide (DMF)), has the same protons and carbons as the diepoide 2, as deduced from the nuclear magnetic resonance (NMR) studies. Comparison of the ¹H- and ¹³C-NMR data of 1 with those of 2¹⁰⁾ is informative. The ¹H-NMR methyl singlet signals of 2 at δ 1.88 and 1.81 due to the isopropylidene group are shifted to higher field in the spectrum of 1. Another signal appeared at lower field at δ 5.26 and this signal is coupled with the H₅ proton in the spectrum of 1. The ¹³C-NMR signal of

1 at δ 156.5 appears instead of that at δ 29.1 due to the C(6) allylic methylene in the spectrum of 2. In addition, the signal of 2 at δ 207.0 due to the C(8) ketone moved to δ 156.5 in the spectrum of 1. The olefinic C(11) signal of 2 at δ 137.4 shifted to higher field at δ 86.7 (allyl alcoholic carbon) in the spectrum of 1.

Based on the above data, one can deduce that 1 probably has an oxirane moiety and a 6,7-*endo* double bond, providing that the C(8) ketone and a hydroxyl group at C(11) are condensed, instead of the 7,11-*exo* double bond in 2.

Dreiding models showed that, while the C(8) ketone is difficult to condense with a C(11)-hydroxyl group to form a hemiacetal oxetene, a C(11)-hydroperoxyl group seems to be better suited for forming a 1,2-dioxalane.

A direct X-ray analysis of 1 was carried out in order to determine its relative stereostructure, which was established to be as shown in Fig. 1. The ten-membered ring in 1 is fused in *trans*-relationship with two oxirane moieties at

Chart 1

Fig. 1. Stereoview of Wenjine (1)

C(1)–C(10) and C(4)–C(5), and a hemiacetal 1,2-dioxalane is attached at the C(7) and C(8) positions. Wenjine (**1**) has a relatively stable diepoxy peroxyhemiacetal moiety, which seems to be rare in nature.

In order to estimate the absolute configuration, stereospecific transformation of (4*S*,5*S*)-(+)-germacrone-4,5-epoxide (**3**)⁸ to **1** was undertaken.

Oxidation of **3** with *m*-chloroperbenzoic acid (MCPBA) provided (1*S*,10*S*), (4*S*,5*S*)-germacrone-1(10),4-diepoxy (**2**) in 86% yield accompanied with a trace amount of germacrone-1(10),4,7(11)-triepoxide (**4**). Compound **4** has the molecular formula C₁₅H₂₂O₄. The triepoxide structure of **4** was supported by the comparison of the ¹H-NMR data of **4** with those of the diepoxy (**2**). Photo-oxidation of **2** using rose bengal or hematoporphyrin as a photosensitizer gave **1** in 25% yield under controlled conditions.¹⁰

The synthetic wenjine shows the same physical and spectral properties as those of the natural product isolated from *C. wenyujin*.⁷ Thus, the absolute configuration of **1** was unequivocally established to be (1*S*,4*S*,5*S*,8*S*,10*S*).

Experimental

Melting points were determined on a Buchi melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 spectrometer. Infrared (IR) spectra were recorded on a Hitachi EPI-G3 spectrophotometer. Mass spectra (MS) were obtained on a JEOL LMS-D300 spectrometer. ¹H- and ¹³C-NMR spectra were measured with a JEOL GS-270 using tetramethylsilane as an internal standard unless otherwise stated. The coupling patterns are indicated as follows: singlet = s, doublet = d, triplet = t, quartet = q, multiplet = m, and broad = br. The coupling constant *J* values are expressed in hertz (Hz).

Isolation of Wenjine (1) Air-dried and cut rhizomes of *Curcuma wenyujin* (875 g) were percolated with ether (4 l) at 5°C for a week. Evaporation of the solvent *in vacuo* afforded 28.5 g of the ether extract as an oil. This oil was submitted to silica gel column chromatography with a petroleum ether–ether solvent system, and fifteen fractions were collected. The eighth fraction (petroleum ether–ether; 5:4) was evaporated. The residue obtained (100 mg) was purified by silica gel chromatography. Recrystallization of the oil from ether afforded 6 mg of (1*S*,10*S*), (4*S*,5*S*)-germacrone-1(10),4-diepoxy (**2**) (0.0016%) as colorless needles. The twelfth fraction (petroleum ether–ether; 5:6) was evaporated *in vacuo* to yield 150 mg of residue. This material was submitted to repeated silica gel chromatography and the fractions eluted with petroleum ether–ether (10:2) were collected. Evaporation of these fractions yielded a crystalline material. This product was recrystallized from methanol–chloroform (1:1) to afford 8 mg (0.001%) of wenjine (**1**) as colorless prisms.

Wenjine (**1**): Colorless prisms; mp 230–231°C (dec.). [α]_D²⁵ +54.3° (*c* = 0.37, DMF). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3425, 3330 (OH). EI-MS *m/z*: 264 (M⁺ – H₂O). ¹H-NMR (CDCl₃, 400 MHz) δ : 5.26 (1H, d, *J* = 8.2, H₆), 4.08 (1H, d, *J* = 8.3, H₃), 3.26 (1H, br s, –OH), 3.15 (1H, d, *J* = 10.4, H₁), 2.74 (1H, d, *J* = 15.0, H₉), 1.46, 1.43, 1.35, 1.30 (each 3H, s, C₁₁(CH₃)₂, C₄–CH₃, C₁₀–CH₃), 1.41–1.43 (2H, m, H₂, H₃). ¹³C-NMR (CDCl₃) δ : 156.5 (C₈), 120.8 (C₂), 106.9 (C₃), 86.7 (C₄), 62.5 (C₅), 62.0 (C₆), 59.1 (C₇), 59.0 (C₅), 50.6 (C₉), 36.7 (C₁₀), 26.5 (C₁₁), 25.2 (C₁₃), 20.4 (C₁₄), 16.8 (C₁₅).

(1*S*,10*S*), (4*S*,5*S*)-Germacrone-1(10),4-diepoxy (**2**): Colorless needles. mp 84–86°C. [α]_D²³ +69° (*c* = 0.51, CHCl₃). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 256 (1660), 315 (203). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1678, 1645 (enone). CD [θ] (nm): +41900 (250), –7400 (325), –7800 (332). HR-MS *m/z*: 250.3524 for C₁₅H₂₂O₃ (Theor. 250.3528). ¹H-NMR (CDCl₃, 270 MHz) δ : 3.02 (1H, d, *J* = 11.6, H₉), 2.94 (1H, dd, *J* = 10.7, 1.1, H₁), 2.88 (1H, dd, *J* = 12.1, 1.8, H₂), 2.67 (1H, dd, *J* = 11.0, 2.0, H₆), 2.66 (1H, d, *J* = 11.6, H₃), 2.30 (1H, dd, *J* = 12.1, 11.0, H₆), 2.22 (1H, m, H₂), 2.08 (1H, m, H₃), 1.88 and 1.81 (each 3H, s, H₁₂, H₁₃), 1.46 (3H, s, H₁₅), 1.21 (3H, s, H₁₄).

X-Ray Analysis of Wenjine (1) The relative structure of wenjine (**1**) was determined by X-ray analysis. The crystal data were as follows: C₁₅H₂₂O₅; M.W. = 282.3; orthorhombic; space group *P*2₁2₁2₁; lattice constants *a* = 11.542(6), *b* = 14.305(9), *c* = 8.759(2) Å, $\alpha = \beta = \gamma = 90^\circ$, *U* = 1446 Å³, *Z* = 4, *D*_{calc} = 1.296 g cm⁻³. The intensity data were collected on a Philips PW 1100 diffractometer using graphite monochromated CuK α

TABLE I. Atomic Coordinates and Equivalent Isotropic Temperature Factors of Wenjine (**1**)

No.	Atom	<i>x</i> · 10 ⁴	<i>y</i> · 10 ⁴	<i>z</i> · 10 ⁴	<i>B</i> _{eq} (Å ²)
1	C1	6474 (3)	7324 (2)	–2464 (5)	3.8 (0.1)
2	C2	6822 (4)	7580 (3)	–4038 (5)	4.3 (0.1)
3	C3	7485 (4)	8515 (3)	–4119 (5)	3.9 (0.1)
4	C4	6751 (3)	9305 (3)	–3512 (4)	3.2 (0.1)
5	C5	6696 (3)	9393 (2)	–1831 (4)	2.8 (0.0)
6	C6	5673 (3)	9755 (2)	–1009 (4)	2.7 (0.0)
7	C7	5140 (3)	9281 (2)	84 (4)	2.5 (0.0)
8	C8	5501 (3)	8348 (3)	765 (4)	3.3 (0.1)
9	C9	5130 (4)	7459 (3)	–86 (5)	3.9 (0.1)
10	C10	5284 (3)	7414 (2)	–1791 (5)	3.3 (0.1)
11	C11	4089 (3)	9618 (3)	958 (4)	3.4 (0.1)
12	C12	4327 (4)	10511 (3)	1834 (5)	4.9 (0.1)
13	C13	3000 (4)	9691 (4)	6 (6)	5.1 (0.1)
14	C14	5810 (4)	9662 (3)	–4521 (4)	4.0 (0.1)
15	C15	4283 (3)	7767 (3)	–2733 (5)	4.4 (0.1)
16	O1	5727 (3)	6510 (2)	–2308 (4)	5.0 (0.1)
17	O2	7391 (2)	10048 (2)	–2725 (3)	3.7 (0.0)
18	O3	4936 (3)	8381 (2)	2245 (4)	5.5 (0.1)
19	O4	3865 (3)	8843 (2)	1982 (4)	5.7 (0.1)
20	O5	6676 (2)	8268 (2)	1122 (3)	4.2 (0.0)

No.	Atom	<i>x</i> · 10 ³	<i>y</i> · 10 ³	<i>z</i> · 10 ³	<i>B</i> _{eq} (Å ²)
21	HC1	719 (4)	733 (3)	–158 (5)	6.0 (1.0)
22	HC2	735 (4)	705 (3)	–462 (5)	6.0 (1.0)
23	H'C2	620 (3)	761 (2)	–475 (4)	3.0 (1.0)
24	HC3	778 (3)	866 (3)	–529 (4)	4.0 (1.0)
25	H'C3	824 (3)	847 (3)	–341 (4)	5.0 (1.0)
26	HC5	719 (3)	894 (2)	–120 (4)	2.0 (1.0)
27	HC6	535 (3)	1039 (2)	–132 (4)	4.0 (1.0)
28	HC9	555 (4)	696 (3)	46 (5)	6.0 (1.0)
29	H'C9	429 (4)	734 (3)	22 (6)	7.0 (1.0)
30	HC12	500 (4)	1049 (3)	261 (6)	7.0 (1.0)
31	H'C12	442 (3)	1106 (3)	101 (5)	5.0 (1.0)
32	H'C12	364 (4)	1071 (3)	268 (6)	8.0 (1.0)
33	HC13	237 (4)	985 (3)	71 (5)	7.0 (1.0)
34	H'C13	301 (4)	1024 (3)	–81 (6)	8.0 (1.0)
35	H'C13	283 (4)	909 (3)	–60 (5)	6.0 (1.0)
36	HC14	526 (4)	917 (3)	–506 (5)	6.0 (1.0)
37	H'C14	542 (4)	1010 (3)	–416 (5)	6.0 (1.0)
38	H'C14	615 (4)	990 (3)	–558 (5)	8.0 (1.0)
39	HC15	408 (4)	837 (3)	–248 (5)	6.0 (1.0)
40	H'C15	432 (4)	770 (3)	–393 (5)	6.0 (1.0)
41	H'C15	363 (4)	746 (3)	–249 (5)	6.0 (1.0)
42	HO5	687 (5)	887 (4)	161 (6)	10.0 (2.0)

Equivalent positions:

<i>x</i>	<i>y</i>	<i>z</i>
1/2 – <i>x</i>	– <i>y</i>	1/2 + <i>z</i>
1/2 + <i>x</i>	1/2 – <i>y</i>	– <i>z</i>
– <i>x</i>	1/2 + <i>y</i>	1/2 – <i>z</i>

radiation. We used 1391 reflections up to 155° (2 θ). The structure was solved by the MULTAN program and refined by the least-squares method with the block-diagonal matrix approximation. The final *R* value was 0.050 including 22 hydrogen atoms with isotropic temperature factors.

Synthesis of (1*S*,10*S*), (4*S*,5*S*)-Germacrone-1(10),4-diepoxy (2**) and (1*S*,10*S*), (4*S*,5*S*), (7*S*,11*S*)-(–)-1(10),4,7(11)-Triepoxide (**4**) from (4*S*,5*S*)-(+)-Germacrone-4-epoxide (**3**)** A mixture of (4*S*,5*S*)-(+)-germacrone-4,5-epoxide (**3**) (500 mg) isolated from *C. wenyujin*,⁷ and MCPBA (400 mg) in 4 ml of CH₂Cl₂ was stirred at room temperature for 24 h. The reaction mixture was washed twice with saturated Na₂SO₄. The organic layer was concentrated under reduced pressure and the residue was subjected to column chromatography over silica gel with a gradient system of petroleum ether and ether to afford 450 mg of **2** (86%) and 20 mg of **4** (4%). **4**: C₁₅H₂₂O₄; colorless powder. mp 138–140°C; [α]_D²⁰ +228° (*c* = 0.90, CHCl₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1705 (C=O). CI-MS *m/z*: 267 (MH⁺). EI-MS *m/z*: 266 (M⁺). HR-MS *m/z*: 266.3595 for C₁₅H₂₂O₄ (Theor. 266.3522). ¹H-NMR (CDCl₃, 270 MHz) δ : 3.07 (1H, d, *J* = 9.9), 3.04 (1H,

d, $J=10.6$), 3.00 (1H, dd, $J=10.3, 0.7$), 2.43 (1H, d, $J=10.6$), 2.25—2.15 (2H, m), 2.10 (1H, dd, $J=13.9, 1.1$), 1.98 (1H, dd, $J=13.9, 2.0$), 1.44, 1.42, 1.19, 1.14 (each 3H, s), 1.28—1.35 (2H, m).

Synthesis of Wenjine (1) from (1S,10S),(4S,5S)-Germacrone-1(10),4-diepoxy (2) A solution of **2** (15 mg) and rose bengal (1 mg) in EtOH (12 ml) was irradiated with a bank of tungsten lamps (200 W $\times$ 3) under bubbling with oxygen for 70 h. The reaction mixture was concentrated under reduced pressure, and the residue was subjected to silica gel column chromatography, wherein was obtained 4 mg (25%) of wenjine (**1**). When hematoporphyrin was used as the sensitizer, the photooxygenation for only 10 h afforded 4 mg (25%) of **1**, mp 230—231 °C.¹⁰⁾ Irradiation for one week was required to provide the same yield of **1** when eosine was used. **1**: ¹³C-NMR (CDCl₃) δ : 207.0 (C₈), 137.4 (C₁₁), 133.8 (C₇), 63.9 (C₄), 61.2 (C₁), 60.0 (C₄), 57.4 (C₁₀), 54.4 (C₉), 35.5 (C₃), 29.1 (C₆), 22.8 (C₂), 22.6 (C₁₂), 20.6 (C₁₃), 17.2 (C₁₅), 15.4 (C₆).

(±)-Wenjine from (±)-Germacrone-1(10),4-epoxide (±)-Germacrone-1(10),4-epoxide (15 mg) was photooxygenated according to the same procedure as described above for the synthesis of wenjine (**1**), giving 3 mg (17%) of (±)-wenjine. mp 244—246 °C.

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- 8) a) M. Yoshihara, H. Shibuya, E. Kitano, K. Yanagi and I. Kitagawa, *Chem. Pharm. Bull.*, **32**, 2059 (1984); b) (4S,5S)-Germacrone-4,5-epoxide (**3**) in their paper would be better designated as (4S,5S)-germacrone-4-epoxide for comparison with compounds, e.g. (1S,10S),(4S,5S)-germacrone-1(10),4-diepoxy (**2**) and (1S,10S),(4S,5S),(7 ξ ,11 ξ)-germacrone-1(10),4,7(11)-triepoxy (**4**), in this paper.
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- 10) The photosensitized reaction of **2** (32 mg) using hematoporphyrin (1 mg) was conducted for 40 h to afford 9-hydroxygermacrone-1(10),4-diepoxy (**5**) (5 mg, 5%) together with **1** (2 mg, 6%). **5**: C₁₅H₂₂O₅, mp 179—180 °C. $[\alpha]_D^{20} + 5.98$ ($c=1.00$, CHCl₃). IR $\nu_{\max}^{\text{KBr}}$ (cm⁻¹): 3435 (OH), 1682, 1632 (enone). CI-MS m/z : 276 ($M^+ - O$). ¹H-NMR (CDCl₃, 270 MHz) δ : 3.73 (1H, s), 3.18 (1H, dt, $J=10.6, 2.6$), 3.08 (1H, dd, $J=13.6, 1.8$), 3.07 (1H, dd, $J=9.2, 1.8$), 2.33 (1H, d, $J=2.5$), 2.28 (1H, dq, $J=5.9, 2.9$), 2.08 (1H, dd, $J=2.0$).