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THE ABSOLUTE STEREOSTRUCTURE OF (4S,5S)-(+)-GERMACRONE 4,5-EPOXIDE FROM ZEDOARIAE RHIZOMA CULTIVATED IN YAKUSHIMA ISLAND

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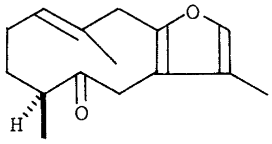
(4S,5S)-(+)-Germacrone 4,5-epoxide (**2**) has been isolated as a characteristic constituent from Zedoariae Rhizoma, the air-dried zedoary cultivated in Yakushima Island in Japan, and the absolute stereostructure (**2**) has been determined from the spectral properties, X-ray analysis, and CD spectra. The epoxide (**2**) seems to be a biogenetically key compound for various germacrane-sesquiterpenoids in zedoary.

KEYWORDS —— Zedoariae Rhizoma; (4S,5S)-(+)-germacrone 4,5-epoxide; germacrane X-ray analysis; α,β-unsaturated ketone CD; β,γ-unsaturated ketone CD

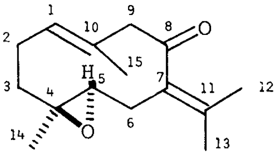
During the course of chemical studies on bioactive constituents of naturally occurring drug materials, we have recently elucidated the absolute stereostructure of furanogermenone (**1**),[1)] which was isolated from Zedoariae Rhizoma (imported from China) as an antihepatotoxic principle for CCl_4-induced liver lesion in mice.[2)] As a continuing study, we have comparatively investigated the chemical constituents of Zedoariae Rhizoma of various origins (e.g. from China, Taiwan, and Yakushima Island of this country) by means of GC-MS.[3)] We have found that Zedoariae Rhizoma prepared from zedoary cultivated in Yakushima Island characteristically contains (4S,5S)-(+)-germacrone 4,5-epoxide (**2**). This paper deals with elucidation of the absolute stereostructure of **2**.

The n-hexane soluble portion, which was obtained by n-hexane-MeOH partition of the MeOH extract of the rhizome, was subjected to centrifugal liquid chromatography and preparative HPLC to afford (4S,5S)-(+)-germacrone 4,5-epoxide (**2**), $C_{15}H_{22}O_2$,[4)] mp 59-60°C (colorless prisms from n-hexane), $[\alpha]_D^{16}$ +399° (c=1.05, $CHCl_3$), in 0.12% yield from the rhizome.

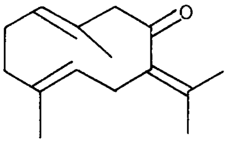
The IR spectrum of **2** showed the presence of an α,β-unsaturated ketone moiety (1672, 1652 cm^{-1}) and an epoxide moiety (840 cm^{-1}), while the UV spectrum (λ_{max}^{MeOH} 239 nm, ε=4500) suggested that the α,β-unsaturated ketone moiety is non-planar. The ^1H-NMR analysis (90 MHz,$CDCl_3$) including the decoupling experiments (Table I) together with the ^{13}C-NMR analysis (22.5 MHz, $CDCl_3$, Table II) in comparison with the data for germacrone (**3**)[5)] led us to conclude that **2** might be either germacrone



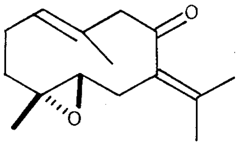
furanogermenone (1)



(4S,5S)-
(+)-germacrone 4,5-epoxide (2)



germacrone (3)



(-)-germacrone
4,5-epoxide (4)

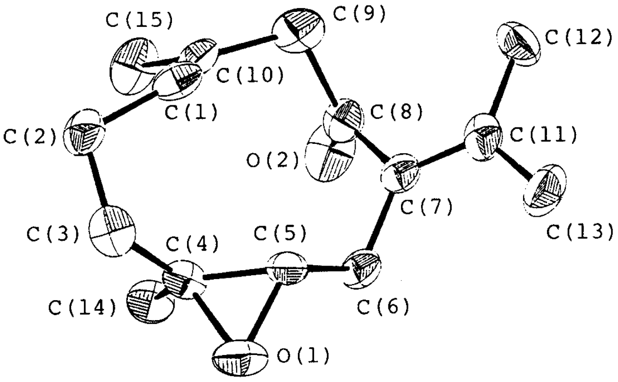


Fig. 1

Table I. ^{1}H-NMR Data for 2 (δ in $CDCl_3$, J values in Hz)

	at 90 MHz	at 300 MHz
1-H	5.22(m)	5.21(br d,J=ca.9.6)
$2\text{-}H_2$	a	2.13,2.26(each m)
3α-H	a	2.1～2.3(m)
3β-H	1.15(m)	1.15(ddd,J=12.0,12.0,7.0)
5-H	2.45(dd,J=2,11)	2.44(dd,J=2.4,10.8) $\xrightarrow{\text{irr. at 2.06}}$ d(J=2.4), $\xrightarrow{\text{irr. at 2.87}}$ d(J=10.8)
6α-H	a	2.06(dd,J=10.8,14.4) $\xrightarrow{\text{irr. at 2.44}}$ d(J=14.4), $\xrightarrow{\text{irr. at 2.87}}$ d(J=10.8)
6β-H	2.89(br d,J=ca.14)	2.87(br d,J=ca.14.4) $\xrightarrow{\text{irr. at 2.06}}$ br s, $\xrightarrow{\text{irr. at 2.44}}$ d(J=14.4)
$9\text{-}H_2$	3.03,3.44(ABq,J=10)	3.02,3.44(each br s)
$4\text{-}CH_3$	1.04(s)	1.04(s)
$10\text{-}CH_3$	1.74(br s)	1.73(br s)
$11\text{-}(CH_3)_2$	1.83(6H,s)	1.82,1.83(each s)

a: These signals were observed as a combined four-proton multiplet in δ2.0～2.4.

Table II. ^{13}C-NMR Data for 2[a] and 3 (δc in $CDCl_3$)

Carbon	2	3	Carbon	2	3	Carbon	2	3
1	129.6(d)	132.8	6	29.6(t)	29.3	11	134.3(s)	137.5
2	24.5(t)	24.2	7	126.6(s)	129.5	12	20.3(q)	19.9
3	37.6(t)	38.2	8	204.4(s)	207.9	13	22.7(q)	22.3
4	60.5(s)	126.9	9	55.4(t)	56.0	14	15.8(q)	15.6
5	64.3(d)	125.6	10	133.7(s)	135.1	15	16.9(q)	16.8

a) Abbreviations given in parentheses indicate the off-resonance signal patterns.

Table III. UV and CD Data for 2

UV λ_{max} (MeOH)	CD (MeOH)
310 nm (ε=600) ($n \rightarrow \pi^*$)	$[\Theta]_{345}$ 0, $[\Theta]_{308}$+15000 (pos.max.), $[\Theta]_{275}$+4600 (pos.min.)
239 nm (ε=4500) ($\pi \rightarrow \pi^*$)	$[\Theta]_{254}$+15000 (pos.max.), $[\Theta]_{239}$ 0, $[\Theta]_{227}$-13000 (neg.max.), $[\Theta]_{220}$ 0

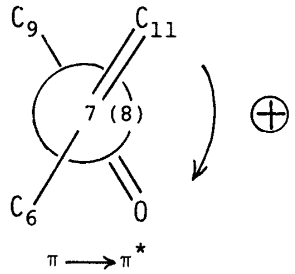


Fig. 2

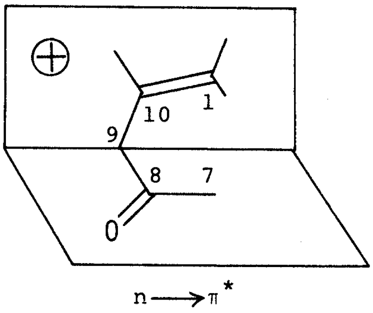


Fig. 3

4,5-epoxide or germacrone 1,10-epoxide. The 4,5-epoxide structure was supported by the 2D ^{1}H-NMR analysis (300 MHz, $CDCl_3$), thus substantiating the plane structure of 2.

In regard to germacrone 4,5-epoxide, (-)-germacrone 4,5-epoxide (4) was isolated earlier from Asarum caulescens Maxim.,[6] however the the absolute stereostructure was not yet unequivocally determined.[7]

To elucidate the relative stereostructure of (4S,5S)-(+)-germacrone 4,5-epoxide (2), X-ray analysis was undertaken first. The crystal used for the X-ray analysis had dimensions of ca. 0.3 x 0.3 x 0.4 mm. The crystal system, cell dimensions, and space group were determined using an Enraf-Nonius CAD 4 diffractometer. Crystal data are: $C_{15}H_{22}O_2$, M.W.=234.34, orthorhombic, space group $P2_12_12_1$, a=8.360(2), b=27.225(4), c=6.234(1) Å , Z=4, Dx=1.093 g cm^{-3}. Intensities were collected on the diffractometer, using graphite monochromated Mo Kα radiation. A total of 1476 reflections were measured to a maximum 2θ of 50° using the ω/2θ scan technique. The structure was solved by using the MULTAN 11/82 system. The refinement of atomic parameters were carried out by fullmatrix least-squares calculations. Most of the hydrogen atoms were located in a difference Fourier synthesis, except some of those associated with the methyl groups which were not included in calculation. The final R value was 0.067 for 617 reflections ($I > 2\sigma(I)$). The molecular structure of the epoxide (**2**) and the numbering of atoms are shown in Fig. 1.

Finally, the absolute stereostructure of **2** has been determined by CD analysis (Table III). Thus, the combination of a positive first Cotton effect at 254 nm and a negative second Cotton effect at 227 nm, which were attributable to the $\pi \rightarrow \pi^*$ transition of the α,β-unsaturated ketone moiety,[8] indicated (+)-chirality between the 7(11)-double bond and the 8-ketone group (Fig. 2). In the $n \rightarrow \pi^*$ transition region, a strong positive Cotton effect was unexpectedly observed at 308 nm. This would be reasonably attributable to the β,γ-unsaturated ketone moiety in **2** by application of the octant rule,[9] in which the 1(10)-double bond was presumed to be the most influential substituent (Fig. 3).

Based on the above-mentioned evidence, the absolute stereostructure of (4S, 5S)-(+)-germacrone 4,5-epoxide (**2**) has been established. This epoxide seems to be an important key compound in the biogenesis of various germacrone-type sesquiterpene metabolites in Zedoariae Rhizoma.

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