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THE STEREOSTRUCTURE OF WENJINE AND RELATED (1S,10S),(4S,5S)-GERMACRONE-1(10),4-DIEPOXIDE ISOLATED FROM CURCUMA WENYUJIN[1)]

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A novel superoxidized sesquiterpenoid named wenjine (1) and germacrone diepoxide (2) with the germacrone epoxide (3) were isolated from the essential oil of the air-dried rhizoma of Curcuma wenyujin. The stereostructure of 1 was determined on the basis of its spectral data and X-ray diffraction. The absolute structure of 2 was also elucidated based on the spectral data and the transformation from 3.

KEYWORDS —— Curcuma wenyujin; wenjine; (1S,10S),(4S,5S)-germacrone-1(10),4-diepoxide; (4S,5S)-germacrone-4,5-epoxide; X-ray diffraction; peroxy hemiacetal; 1,2-dioxalane

In our ongoing studies on the biologically active constituents, especially of antitumor activity in a Chinese medicinal plant, Curcuma wenyujin (Zingiberaceae),[3-5)] we have reported on the isolation and structure of curcumol,[6)] curdione,[7-11)] curcumalactone,[8,9,11)] and neocurdione[9,10,12,13)] from the essential oil of the air-dried rhizoma. We now describe the structure of a unique superoxidized germacranoid, referred to wenjine (1) and (1S,10S),(4S,5S)-germacrone-1(10),4-diepoxide (2) except (1R,10R)-epoxy-(-)-1,10-dihydrocurdione.[14)]

An extract of the air-dried rhizoma was successively eluted by silica gel chromatography using a gradient solvent of pet. ether and ether as reported previously.[14)] After separation of the above-mentioned earlier major constituents, some polar middle fractions such as fraction 6 (solvent ratio 2:1), fraction 8 (5:4) and the more polar later fraction 12 (5:6) afforded 2 (0.0016%), 3 (0.023%) and 1 (0.001%), respectively.

Monoepoxide (3): mp 56-58°C (pet. ether); $[\alpha]_D^{25}$ +119.9° ($CHCl_3$, c 0.95);[15)] The ^1H-NMR and ^{13}C-NMR spectral data together with the physical constants indicate that 3 should be (4S,5S)-epoxy-(+)-4,5-dihydrogermacrone, i.e. "(4S,5S)-germacrone-4,5-epoxide" isolated from C. zedoaria,[16)] but not "germacrone-4,5-epoxide" containing the authentic (4R,5R)-(-)-enantiomer[15)] isolated from Asarum caulescens (Aristolochiaceae).[17)]

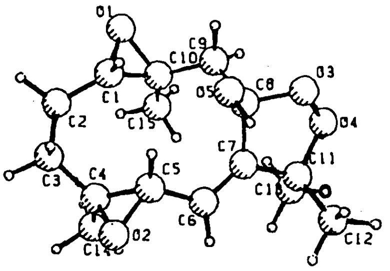
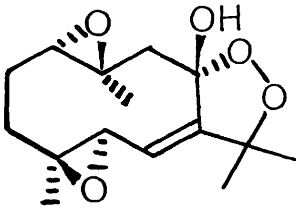
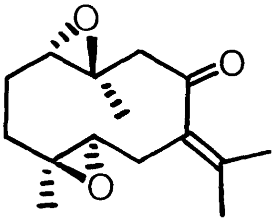


Fig. 1. Perspective View of Wenjine(1)



1



2

Diepoxide (2): mp 84-86°C (pet. ether/ether);[18) $[\alpha]_D^{23}$ +69° ($CHCl_3$, c 0.51); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 3.02 (1H, d, J = 11.62, H_9), 2.94 (1H, dd, J = 10.65, 1.10, H_1), 2.88 (1H, dd, J = 12.10, 1.80, H_5), 2.67 (1H, dd, J = 10.99, 2.00, H_6), 2.66 (1H, d, J = 11.62, H_9), 2.30 (1H, dd, J = 12.10, 10.99, H_6), 2.22 (1H, m, H_2), 2.08 (1H, m, H_3), 1.88 and 1.81 (each 3H, s, H_{12}, H_{13}), 1.46 (3H, s, H_{15}), 1.21 (3H, s, H_{14}); UV λ_{max}^{MeOH} nm (ε): 256 (1660) and 315 (203); IR ν_{max}^{KBr} cm^{-1}: 1678, 1645 (enone); CD [θ] (nm): +41900 (250), -7400 (325), -7800 (332); HR-MS m/z: 250.1524 for $C_{15}H_{22}O_3$ (theor. 250.3528). The above data suggest that the composition of 2 has one more oxygen than 3 and the α,β-unsaturated ketone moiety still remains in its molecule. From the ^{1}H-NMR and ^{13}C-NMR spectral comparison between 2 and 3, 2 appeared to be a 1(10)-epoxide of 3. The relative stereo-chemistry of 2 was deduced to be in cis-relationship with respect to the oxylanes, based on the observation of 3.6% NOE between the C(4)-Me and C(10)-Me. Since the chemical transformation from a possible biogenetic precursor 3 to 2 was achieved by epoxidation of 3 with MCPBA (1 eq) in CH_2Cl_2 at room temperature in a yield of 86%,19) the absolute structure was unequivocally established to be (1S,10S), (4S,5S)-diepoxy-(+)-1,4,5,10-tetrahydrogermacrone, which is now named (1S,10S), (4S,5S)-germacrone-1(10),4-diepoxide.18) The chiral data of this authentic, naturally-occurring diepoxide (2) is obviously different from those of the cis- and trans-isomer of "1,10;4,5-diepoxygermacrone"20) obtained by the epoxidation of "germacrone-4,5-epoxide".17) They also showed a discrepancy with those of each enatiomer of the cis-isomer ("1,10;4,5-dioxidogermacr-7(11)-en-8-one") obtained from the microbial oxygenative transformation from germacrone.21) The absolute configuration of each chiral diepoxide, regarded as the antipode (mp 123.5-124.5°C; $[\alpha]_D$——) deduced from their RD analysis, was not based on the real sign of the Cotton effect.21b) In addition, this bio-oxygenetic asymmetric induction appeared to be not enatioselective.21) These data indicate no precedence of the diepoxide having the abolute configuration of 2.

The third highly oxygenated diepoxide, wenjine (1) consists of colorless prisms of mp 230-231°C (dec) ($CHCl_3$/MeOH); $[\alpha]_D^{22}$ +54.3° (DMF, c 0.37); IR ν_{max}^{KBr} cm^{-1}: 3425, 3330 (OH); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.26 (1H, d, J = 8.42, H_6), 4.08 (1H, d, J =8.25, H_5), 3.26 (1H, brs, -OH), 3.15 (1H, brd, J = 10.44, H_1), 2.74 (1H, d, J = 15.00, H_9), 2.22-2.17 (2H, m, H_2, H_3), 2.09 (1H, d, J = 15.00, H_9), 1.46, 1.43, 1.35 and 1.30 (each 3H, s, H_{12}, H_{13}, H_{14}, H_{15}), 1.43-1.41 (2H, m, H_2, H_3). Its ^{1}H-NMR and ^{13}C-NMR data compared with those for 2 and 3 indicate that

1 has the same number of protons and carbons as 2 and 3. In addition to the ^{1}H-NMR methyl singlets due to the isopropylidene group at δ 1.88 and 1.81 in 2 moved towards a high field in 1, a signal appeared downward at δ 5.26 coupling with H_5 with a J value of 8.25 Hz. On the other hand, the ^{13}C-NMR signal at δ 120.8 in 1 appeared instead of that at δ 29.1 for the C(6) arylic methylene in 2, and the signal at δ 207.0 for the C(8) ketone in 2 moved upward at δ 156.5 in 1 in relation to the disappearance in 1 of the IR carbonyl absorption (1678 cm^{-1}) in 2. In addition, the olefinic C(11) signal at δ 137.4 in 2 shifted to a higher field at δ 86.7 for an aryl alcoholic carbon. Based on the above data, one can probably deduce that wenjine has a structure, including the oxirane moiety, related to 2 except for the migration of the exo $\Delta^{7(11)}$ double bond to the endo form, providing the condensation between the ketone at C(8) and a hydroxyl group which might be attached to C(11). Dreiding models show that, while the C(8)-ketone is difficult to condense with a C(11)-hydroxyl group to form hemiacetal oxetane, a C(11)-hydroperoxyl group seems to be better suited for forming a 1,2-dioxalane. The cyclic hemiacetal formation would not be an extremely unreasonable event, but may be possible by such a specific biogenetic pathway as photosensitized oxygenation. These data agreed with those of the MS analysis which gave a peak at m/z 264, a dehydrate ion equivalent to $C_{15}H_{20}O_4$, suggesting that wenjine has MW 284 with the composition $C_{15}H_{22}O_5$ which builds up a structure expressed in 1. In order to prove the above deduction and to determine the relative configuration, a direct X-ray analysis was carried out. The crystal data are: $C_{15}H_{22}O_5$; MW 282.3; orthorhombic; space group $P2_12_12_1$; lattice constants *a* = 11.542(6), *b* = 14.305(9), *c* = 8.759(2) Å, $\alpha = \beta = \gamma = 90°$; U = 1446 $Å^3$, z = 4; D_{calc} = 1.296 gcm^{-3}. The intensity data were collected on a Phillips PW 1100 diffractometer using graphite monochromated CuKα radiation. We used 1391 reflections up to 155° (2θ), the structure was solved by the MULTAN program and refined by the method of least-squares with block-diagonal matrix approximations. The final R value was 0.050 including 22 hydrogen atoms with isotropic temperature factors. The relative configuration of wenjine and its crystalline conformation are depicted in Fig. 1.

Figure 1 shows that, as deduced above, the ten-membered ring in 1 is trans-fused with the two oxirane at 1(10) and 4(5), and further fused with a hemiacetal 1,2-dioxalane at the C(7) and C(8) positions. The ornamental fusion of the three rings containing oxygen atoms with the central middle ring contributes a rigidity of the whole molecule in comparison with the similar congenic carbocycles, such as germacrone,[21)] curdione[7,10)] and neocurdione.[10,12)] The C(4)- and C(10)-methyl group are oriented downward parallel in the opposit direction from the hemiacetal hydroxyl group with respect to the gross plane of the ten-membered ring.

Wenjine has a relatively stable diepoxy peroxyhemiacetal structure which is rare and unique in nature. The possible biogenetic pathway from germacrone to 1 via 3 and 2 can be plausibly explained based on a rationale of the known absolute configuration of 3[16)] in relation to the coexistence of 1, 2 and 3 together with germacrone.[9)] Concerning the configuration at C(1) and C(10), the diversity in the stereochemistry of (1*S*,10*S*) in 1 and 2 compared with that of (1*R*,10*R*)-epoxy-(-)-1, 10-dihydrocurdione[22)] isolated in the same plant is of interest from the biogenetic point of view. A further study of the definite absolute configuration of 1 and a synthetic approach to it is now in progress.

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19) In relation to Notes 15 and 18, 2% of the above-mentioned racemic diepoxide was separated first by recrystallization from pet. ether. Then the optically active diepoxide (2) was obtained in a pure state.
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