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CONFIGURATION AND CONFORMATION OF TAIBAIHENRYIIN T ISOLATED FROM *Phlomis umbrosa*

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Taibaihenryiin T was isolated for the first time from Phlomis umbrosa *Turcz, and its structure was elucidated on the basis of IR and NMR spectra analysis. Its molecular configuration, conformation, and crystal structure were also characterized by X-ray structure analysis. The infrequency of the C–O–O–C group is manifested in this molecular configuration, and hydrogen bonding assembles the molecules into a three-dimensional networking structure in the crystal.*

Keywords: taibaihenryiin T, *Phlomis umbrosa,* X-ray structure analysis, C–O–O–C group.

Previously some compounds with various biological activities, such as antitumor [1], anti-inflammatory, immunosuppressive, antimutagenic [2], free radical scavenging [3], and antimicrobial [4], were discovered from medicinal plants of the genus *Phlomis*, and they have been shown to contain different classes of glycosides comprising iridoids, flavonoids, phenylpropanoids, phenylethanoids, and diterpenoids [5, 6]. To find new biologically active compounds, recently we continued to investigate the chemical constituents of *Phlomis umbrosa* Turcz collected on the Taibai Mountain of Shaanxi Province in China [7, 8], which led to the first isolation of taibaihenryiin T from this plant. Taibaihenryiin T (**1**) is the specific endoperoxide also named *ent*-4β-methyl-6β,7β,11α-trihydroxy-19α-acetoxy-7α,10α-peroxy-simlikaur-16-en-15-one. At first, we studied the molecular structure and stereochemistry of **1** by chemical means, which is intricate work including IR and NMR spectra [9], but those methods did not give an unambiguous assignment for the configuration and conformation of **1**, so we performed a single-crystal X-ray structure analysis (XSA) of taibaihenryiin T. Figure 1 shows the molecular structure of taibaihenryiin T from X-ray diffraction analysis. Taibaihenryiin T belongs to the minor norditerpenoid constituents in plant. Its molecular configuration shows the infrequency of the C–O–O–C group, indicating its consuming inhibitive effect on malarial disease and that it may be a crucial antimalarial precursor.

The fusion of four rings (A, B, C, D) is evident in the molecular of taibaihenryiin T, and only the D ring is a five elements ring as judged from NMR spectra and the bond lengths and angles obtained from XSA. The B ring has the specific endoperoxide (C–O–O–C group) attached between C7 and C10. In this molecule, the A ring has the chair conformation, C1, C2, C4, and C5 are coplanar, and C3 and C6 are at both sides of the plane figure. The B ring has the boat conformation, and the two fore carbons of the boat conformation are C7 and C10 interlinked by the peroxide group, forming a dihedral angle and at the same time deviating from the coplane of C5, C6, C8, and C9. The C ring has the boat conformation, C8, C9, C12, and C13 are coplanar, and C11 and C14 are the two fore carbons. The boat conformation B ring has almost uniform dimensional orientation with the C ring. The D ring has an envelope conformation; C14 is the packet of the envelope conformation D ring and deviates from the coplane of C8, C15, C16, and C17 because of their characteristic alkene C=C bond and ketonic C=O bond. There are nine asymmetric carbon atoms C4, C5, C6, C7, C8, C9, C10, C11, and C13 in the molecule of **1**; their configuration is seen clearly in Fig. 1. The methyl linking at C4 has β-equatorial orientation, and the two hydroxyls at C6 and C7 also have β-equatorial orientation. The hydroxyl at C11 has α-axial orientation, and the endoperoxide bond linking at C7 and C10 also has α-axial orientation.

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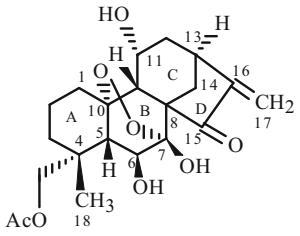
TABLE 1. Crystal Data and Structure Refinement for Taibaihenryiin T, $C_{21}H_{28}O_8$

Formula weight	408.43	θ Range for data collection	1.82–25.10°
Temperature	296 (2) K	Limiting indices	$-9 \le h \le 10$, $-12 \le k \le 8$, $-26 \le I \le 26$
Wavelength	0.71073 Å	Reflections collected/unique	10015/3581 [R(int) = 0.0393]
Crystal system	Orthorhombic	Completeness to θ = 25.10°	100.0%
Space group	$P2_12_12_1$	Absorption correction	multi-scan
Unit cell dimensions	a = 8.451 (4) Å	Max. and min transmission	0.9812 and 0.9641
	b = 10.615 (5) Å	Refinement method	Full-matrix least-squares on F^2
	c = 22.371 (10) Å	Data/restraints/parameters	3581/0/268
	$\alpha = \beta = \gamma$ = 90.00°	Goodness-of-fit on F^2	0.978
Volume	2007.0 (16) $Å^3$	Final R indices [I>2 σ (I)]	RI = 0.0510, ωR2 = 0.1352
Z, calculated density	4.1:352 $Å \cdot m^{-3}$	R indices (all data)	RI = 0.0767, ωR2 = 0.1468
Absorption coefficient	0.103 mm^{-1}	Absolute structure parameter	0.6 (16)
F (000)	872	Extinction coefficient	0.0031 (15)
Crystal size	0.36 mm × 0.25 mm × 0.18 mm	Largest diff. peak and hole	0.267 and –0.443 $e \cdot Å^{-3}$



1

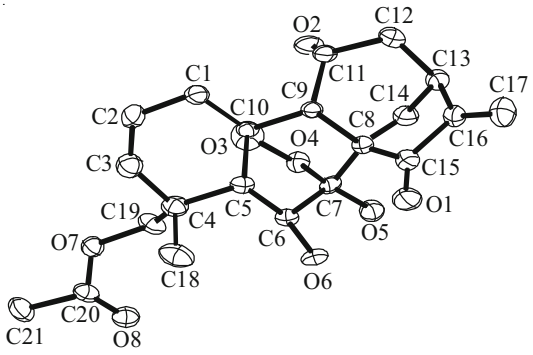


Fig. 1. The molecular structure of taibaihenryiin T showing 50% probability displacement ellipsoids.

The molecules of the title compound are packed in a three-dimensional structure in the crystal by two species of H-bonds. There are two main species of O-H…O hydrogen bonds in the crystal structure; one is between the hydroxyl and the ketone, and the other is between two hydroxyls. The O-H…O hydrogen bonds are between the hydroxyl O5 at C7 and the ketone O8 at C20 of the other molecule [O5…O8 (–x+2, y–1/2, –z +1/2): 2.81 Å, H5…O8: 2.02 Å, angle of O5-H5…O8: 160.7°]. The other O-H…O hydrogen bonds are between the hydroxyl O2 at C11 and the hydroxyl O6 at C6 of the other molecule [O2…O6 (x–1, y, z): 2.75 Å, H2…O6: 1.98 Å, angle of O2-H2…O6: 166.3°]. The O-H…O hydrogen bond of the inner molecule is between the hydroxyl O6 at C6 and the ketone O1 at C15 of the same molecule [O6…O1: 2.68 Å, H6…O1: 1.89 Å, angle of O6-H6…O1: 161.5°]. Thus, one molecule forms four main O-H…O hydrogen bonds with other four different molecules. These O-H…O hydrogen bonds lead to **1** in a double screw chain arranged along the b axis.

The infrequency of the C–O–O–C group in the taibaihenryiin T molecule of this nature compound is interesting. The –O–O– bridge bond interlinks C7 and C10 in the taibaihenryiin T molecule; the bond length of C7-O4 is 1.418 Å, O4-O3: 1.466 Å, and O3-C10: 1.438 Å; the angle of C7-O4-O3 is 113.9°, and O4-O3-C10: 109.4°, showing that the C–O–O–C group exists in the molecule.

EXPERIMENTAL

The tender leaf and branch of *Phlomis umbrosa* Turcz were collected from the Taibai Mountain of Shaanxi Province in China, July 2007, and identified by comparing them with a voucher specimen deposited at the Biology Department of Shaanxi Normal University, Xi′an, China. All chemicals used were of analytical reagent grade and were used directly. Dry powders (4.3 kg) of the leaf and branch of *Phlomis umbrosa* Turcz were extracted for 36 h with 75% ethanol (8.0 L) three times. The ethanol-soluble portion of the extract was dried on a rotary evaporator under reduced pressure and subjected to silica-gel (200~300 mesh, 3.1 kg) column chromatography (1400 cm × 17.0 cm). The column was eluted with $CHCl_3$,

$CHCl_3$–Me_2CO (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), and Me_2CO. Each eluent was collected as 500 mL fractions. All components were also purified by the other small silica-gel column. Taibaihenryiin T was eluted and obtained from the fraction with $CHCl_3$–Me_2CO (6:4) and recrystallized from methanol to yield colorless prism crystals (0.39 g).

The infrared spectrum was recorded as KBr pellets on a Nicolet 170SX FT-IR spectrophotometer. The melting point was determined using a WRS-113 digital melting point instrument (the thermometer was not corrected). The ^{1}H NMR, ^{13}C NMR, and 135°DEPT spectra were recorded on a Bruker AM-300 spectrometer with TMS as internal reference and DMSO-d_6 as solvent.

Taibaihenryiin T was assigned the molecular formula $C_{21}H_{28}O_8$, mp 213–215°C (with decomposition). IR (KBr, ν_{max}, cm^{-1}): 3232.37, 2940.36, 1735.70, 1643.39, 1502.57, 1455.52, 1373.24, 1242.19, 1172.91, 1055.90, 1031.34, 991.64, 939.16, 914.12, 889.06, 604.53, 571.97. The data 3232.37 of the IR spectrum shows that the hydroxyl exists in the molecular structure; it is due to the stretching vibration; 1242.19 and 1172.91 are ascribed to the C-O bond, and 914.12~889.06 to the –O–O– bond. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 6.27 (1H, br.s, OH-7α), 5.75 and 5.44 (each 1H, br.s, H_2-17), 4.82 (1H, s, H-6β), 4.36 and 4.16 (each H, d, H_2-19), 4.08 (1H, s, H-11α), 3.70 (2H, m, OH-6α, OH-11β), 3.12 (1H, d, H-13β), 2.86 (H, dd, J = 11.1, H-14α), 2.27 (1H, d, J = 14.7, H-12α), 2.04 (3H, s, OAc), 1.87 (2H, m, H-14β, H-1β), 1.75 (1H, d, J = 12.9, H-3β), 1.55 (1H, d, J = 15.0, H-12β), 1.41 (1H, d, H-9β), 1.31 (4H, m, H-5β, H-3α, H-2β, H-1α), 1.08 (3H, s, Me-18), 0.96 (1H, d, H-2α). ^{13}C NMR (δ, ppm): 29.8 (t, C-1), 18.1 (t, C-2), 35.4 (t, C-3), 37.1 (s, C-4), 61.2 (d, C-5), 72.8 (d, C-6), 95.5 (s, C-7), 59.0 (s, C-8), 53.6 (d, C-9), 68.6 (s, C-10), 64.8 (d, C-11), 40.5 (t, C-12), 34.0 (d, C-13), 26.6 (t, C-14), 210.8 (s, C-15), 153.7 (s, C-16), 116.2 (t, C-17), 28.0 (q, C-18), 66.1 (t, C-19), 171.0 (s, C-20), 21.2 (q, Me-18). The type of carbon atom substitution was determined by a 135°DEPT experiment.

The crystals of taibaihenryiin T for X-ray diffraction analysis were obtained by slow evaporation from a methanol solution after five days at room temperature. The data were collected with graphite-monochromated Mo/K_α radiation (λ = 0.71073 Å) on a Bruker Smart-1000CCD diffractometer at room temperature. The structure was solved using direct methods and refined by full-matrix least-squares techniques. All non-hydrogen atoms were assigned anisotropic displacement parameters in the refinement. All hydrogen atoms were added at calculated positions and refined using a riding model. The structure was refined on F^2 using SHELXTL-97, and the final R index (on F^2) was 0.0510. The crystals used for the diffraction study showed no decomposition during data collection. The crystal data and some details of the structure determination are summarized in Table 1. Data from the X-ray structure analysis were deposited as a CIF-file in the Cambridge crystallographic Data Center (No. CCDC 705658).

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