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Absolute Configurations of Unique Harziane Diterpenes from *Trichoderma* Species

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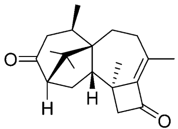
ABSTRACT



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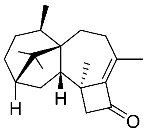

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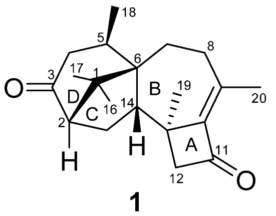
Harzianone (2), a new harziane diterpene, was isolated from an alga-endophytic isolate of *Trichoderma longibrachiatum*. The structure and absolute configuration of 2 were unambiguously identified by NMR and mass spectrometric methods as well as quantum chemical calculations. The absolute configuration of harziandione (1) was supported by calculation of optical rotation, and the structure of isoharziandione was revised to 1 on the basis of ^{13}C NMR data comparison and calculation.

Trichoderma spp. have been suggested as potential biocontrol agents against plant pathogens.[1,2] Harziandione (**1**), containing a unique tetracyclic scaffold (fused four-, five-, six-, and seven-membered carbon rings), was the first harziane diterpene isolated from *T. harzianum*. It exhibited potent antifungal activities against plant pathogens, and the structure was established by NMR, X-ray diffraction, and mass spectrometric methods. However, its absolute configuration was unresolved.[3] Subsequently, a so-called isomer (isoharziandione) of **1** was obtained and identified from *T. viride*, which could also inhibit phytopathogenic fungi.[2] No other reports related to this unique harziane backbone have appeared so far. Fortunately, our recent efforts on an alga-endophytic isolate of *T. longibrachiatum*, obtained from a sample of the marine green alga *Codium fragile*, led to the isolation of another harziane diterpene, harzianone (**2**). The structure and absolute configuration of **2** were unambiguously identified by NMR and mass spectrometric methods as well as quantum chemical calculations. The absolute configuration of **1** was deduced by calculation of optical rotation, and the structure of isoharziandione was revised to **1** based on the calculation of ^{13}C NMR data. The main subjects of this paper are the isolation, structure elucidation, and bioactivity of compound **2** and structure revision of harziandione and isoharziandione by quantum chemical methods.



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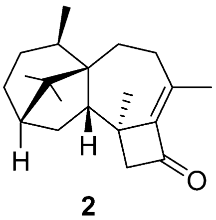

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Compound **2** was obtained as a colorless oil. The molecular formula was determined to be $C_{20}H_{30}O$ on the basis of HREIMS (m/z 286.2304 $[M]^+$, calcd for $C_{20}H_{30}O$, 286.2297), requiring six degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) along with HSQC data displayed four methyl singlets and one methyl doublet. The ^{13}C and DEPT NMR spectra (Table 1) demonstrated the presence of five methyls, six methylenes, three methines, and six nonprotonated carbons. The above NMR data closely resembled those reported for harziandione (**1**) except for the presence of signals for one additional methylene (C-3)

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and the lack of one of the ketone signals (δ_C 214.4, C-3).[3] Accordingly, compound **2** was deduced to be a deoxy derivative at C-3 of **1**. This proposal was supported by the $^1H-^1H$ COSY and HMBC correlations as illustrated in Figure 1.

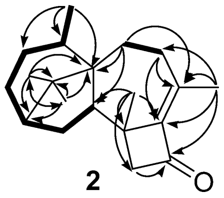

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Figure 1. Key $^1H-^1H$ COSY (solid line) and HMBC (arrow) correlations of **2** (those related to H-7 and H-8 arising from H-7b and H-8b).

The relative configuration of **2** was determined by a NOESY experiment. The NOESY correlations of H_3-16 with H-2 and H-14 suggested a *syn* orientation of them. Hence, ring D was oriented on the opposite face of ring C relative to these protons. The vicinity of H-5 and C-19 was deduced by the NOESY correlation between H-5 and H_3-19. The energy-minimized conformer (Figure 2) was generated by the Dreiding force field in MarvinSketch and further optimized using density function theory (DFT) at the B3LYP/6-31G(d) level in chloroform via Gaussian 09 software,[4] which matched well the above NOESY data. Thus, the relative configuration of **2** was established to be the same as that of **1**.

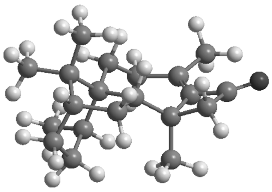

Figure 2. Three-dimensional energy-minimized conformer of **2** in chloroform.

Quantum chemical calculations have been proven to be effective tools in deducing the structures and configurations of natural products. To establish the absolute configuration of **2**, the electronic circular dichroism (ECD) spectrum (Figure 3) was determined, which exhibited a positive Cotton effect at 338 nm (n→π* transition) and a negative Cotton effect at 251 nm (π→π* transition). Overall, 11 possible conformers were generated by the Dreiding force field,[4] the geometries of which were further optimized at the B3LYP/6-31G(d) level in methanol to give just one conformer within a 3 kcal/mol energy threshold from the global minimum. This predominant conformer was subjected to the theoretical calculation of ECD spectrum using the time-dependent DFT method at the B3LYP/6-31G(d) level in methanol with the integral equation formalism variant (IEF) of the polarizable continuum model (PCM).[5] The calculated ECD spectrum (Figure 3) was in good accordance with the experimental one,[6] which indicated the absolute configuration of **2** to be 2*S*,5*R*,6*R*,13*S*,14*S*.

Table 1. ^{1}H and ^{13}C NMR Data for **2** (in $CDCl_3$)[a]

position	δ_H, mult (*J* in Hz)	δ_C
1		46.1, qC
2	1.66, m	42.8, CH
3a	1.31, m	25.7, CH_2
3b	1.94, m	
4a	1.26, m	25.3, CH_2
4b	2.08, m	
5	2.42, m	29.2, CH
6		50.8, qC
7a	1.26, m	30.2, CH_2
7b	1.83, m	
8a	1.26, m	29.3, CH_2
8b	1.89, m	
9		146.5, qC
10		150.0, qC
11		199.4, qC
12a	2.38, d (16.2)	59.9, CH_2
12b	2.53, d (16.2)	
13		40.8, qC
14	2.16, dd (11.4, 8.9)	52.2, CH
15a	1.37, dd (13.3, 8.9)	27.5, CH_2
15b	1.86, m	
16	0.85, s	25.9, CH_3
17	1.04, s	22.5, CH_3
18	1.04, d (7.6)	20.7, CH_3
19	1.49, s	21.6, CH_3
20	2.09, s	22.6, CH_3

[a] Recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively.

In order to confirm the absolute configuration of harziandione (**1**), its specific optical rotation ($[\alpha]_D$) was calculated at the gas-phase B3LYP/6-311++G(2d,2p) level.[7] The original conformational search was also performed using the Dreiding force field,[4] and only one predominant conformer was produced after further optimization with the gas-phase B3LYP/6-31G(d) basis set. The calculated specific rotation (+166.3°) of **1** was close to the reported experimental value (+116.5°),[3] which differed greatly from the calculated $[\alpha]_D$ (−165.9) of its enantiomer. Thus, the absolute configuration of harziandione (**1**) was suggested to be 2*S*,5*R*,6*R*,13*S*,14*S*. Additionally, the deviation (48.9) between experimental $[\alpha]_D$ (+27.5) and calculated $[\alpha]_D$ (+76.4) of **2** was almost the same as that (49.8) of **1**, which further verified the absolute configuration of **1**.

Only a gross structure of isoharziandione was proposed in the literature,[2] which differed from harziandione (**1**)

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(4) Calculator Plugins were used for structure property prediction and calculation, Marvin 5.9.2, 2012, ChemAxon (http://www.chemaxon.com).

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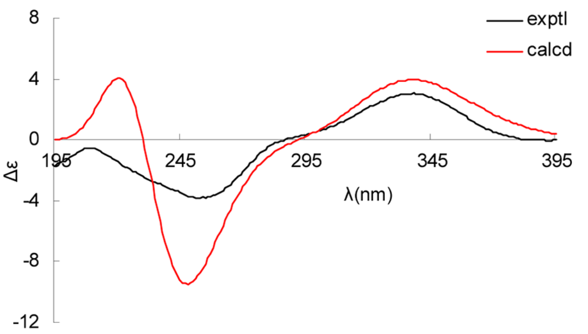

Figure 3. Experimental and calculated ECD spectra of **2** in methanol.

only at the position of the carbonyl group in ring A. However, a detailed comparison of the ^{13}C NMR data reported for isoharziandione with those for harziandione (**1**) revealed that the differences (−0.01 to +0.28) between them were negligible,[2,3] which suggested an identical structure and relative configuration for these two molecules. The upfield signal for a carbonyl group at δ_C 198.02 also suggested a conjugated unit in isoharziandione. In an effort to confirm the above deduction, the ^{13}C NMR data for the reported structure of isoharziandione were calculated with the B3LYP functional and 6-31+G(d,p) basis set in chloroform with tetramethylsilane (TMS) as a reference.[8] The calculated values (Supporting Information) were not in agreement with those reported for isoharziandione,[2] and the large deviations (−10.8, −20.3, and +15.5, respectively) of ^{13}C NMR data for C-9, C-10, and C-12 suggested improper assignments at these positions. On the basis of the above evidence, the structure and relative configuration of isoharziandione were revised to **1**. The conflicting literature specific rotation values (+1.17 reported for isoharziandione and +116.5 for **1**) might arise from sample impurities or could possibly be due to a calculation issue,[2,3] since the values differ by almost exactly 2 orders of magnitude. Although the reason for this difference is not clear, the absolute configurations of **1** and **2** were tentatively deduced to be the same based on biogenic considerations and identical sign (+) of their specific rotations.

Compound **2** was evaluated for antibacterial, antifungal, and brine shrimp toxicity.[9,10] The results showed that **2** inhibited *Escherichia coli* and *Staphylococcus aureus* (inhibitory diameters of 8.3 and 7.0 mm, respectively) at a concentration of 30 μg/disk. In the brine shrimp (*Artemia salina*) toxicity assay, **2** displayed an 82.6% lethality at 100 μg/mL and an LC_{50} value of 23.1 μg/mL. However, no antifungal activities against two phytopathogens (*Colletotrichum lagenarium* and *Fusarium oxysporum*) were found at 30 μg/disk.

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Supporting Information Available. ^{1}H, ^{13}C, DEPT, HSQC, HMBC, $^{1}H-^{1}H$ COSY, NOESY, HREIMS, and IR spectra of compound **2** and the Cartesian coordinates for energy-minimized conformers of **1**, **2**, and the originally proposed structure of isoharziandione. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.