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Two Furanharzianones with 4/7/5/6/5 Ring System from Microbial Transformation of Harzianone

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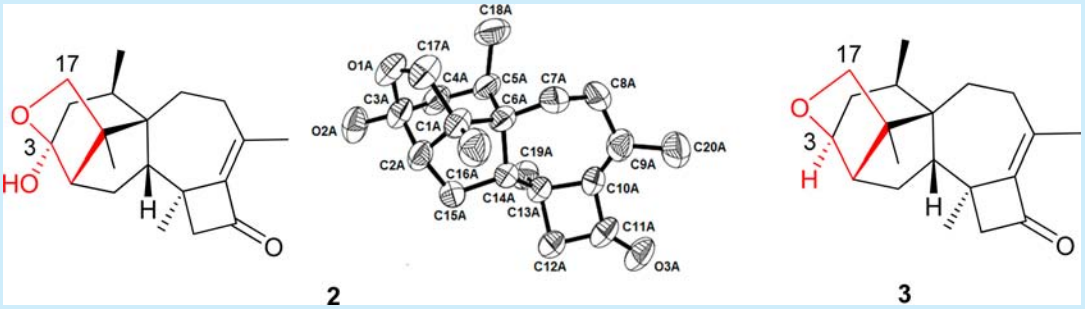
Supporting Information



ABSTRACT: Furanharzianones A and B (**2** and **3**), two new harziane-type diterpenoids with a tetrahydrofuran and unusual 4/7/5/6/5 ring system, were obtained from the microbial transformation of harzianone (**1**) by a bacterial strain *Bacillus* sp. IMM-006. The structures, including the stereochemistry, of the two new compounds were elucidated by extensive spectroscopic analysis. The absolute configuration of **2** was unambiguously determined by single-crystal X-ray diffraction. In addition, a plausible bioconversion pathway was proposed.

Harzianone (**1**), a diterpenoid containing a unique tetracyclic scaffold with fused four-, five-, six-, and seven-membered carbon rings, was first isolated from *Trichoderma longibrachiatum*.[1] The structural identification of **1** led to the structural revision of harziandione[2] and isoharziandione.[3] Subsequently, three harziane-type tetracyclic diterpenes were isolated from *T. atroviridae* UB-LMA[4] as well as trichodermaerin from *T. asperellum*[5] and two harziane diterpenoids from *Trichoderma* sp. Xy24.[6] To the best of our knowledge, these are the only members of the harziane tetracyclic diterpene family reported to date, and fungi in *Trichoderma* genus are the only known producers of this type of compounds. Inspired by the interest in exploring the potential structure diversity of harziane diterpenes with better biological activities, microbial transformation as a powerful approach was employed. After preliminary screening by HPLC−MS analyses, a bacterial strain *Bacillus* sp. IMM-006 with the ability to transform harzianone (**1**) was used for preparative-scale biotransformation. After the standard two-stage fermentation protocol,[7] two new metabolites **2** and **3** with unusual 4/7/5/6/5 ring system including a tetrahydrofuran in the skeleton, along with a known compound harziandione (**4**), were obtained (Figure 1) by a combination of open silica gel column chromatography and semipreparative HPLC.[8] On the basis of IR, HR-MS, 1D-NMR, 2D-NMR, and single-crystal X-ray diffraction analyses, their structures were established as 3α-hydroxy-3β,17-epoxyharzianone (furanharzianone A, **2**, ∼4.2%), 3β,17-epoxyharzianone (furanharzianone B, **3**, ∼1.7%),

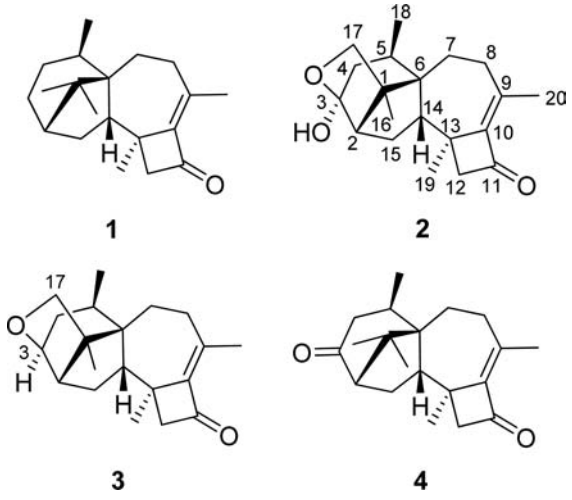


Figure 1. Chemical structures of **1**−**4**.

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and harziandione (**4**, ~5.1%). Herein, we report their isolation, structural elucidation, plausible bioconversion route, and biological activity.

Furanharzianone A (**2**)[9] was obtained as colorless needle crystals (MeOH−H_2O). Its molecular formula, $C_{20}H_{28}O_3$, was established by positive HR-ESI-MS (*m*/*z* 317.2105 $[M + H]^+$, calcd for $C_{20}H_{29}O_3$, 317.2111), corresponding to seven degrees of unsaturation. The ^1H NMR data of **2** were closely similar to those of **1**[1] (Table S1, Supporting Information, SI), except that the H_3-17 signal at δ_H 1.10 (3H, s) in **1** was absent, while an additional oxygenated methylene [δ_H 3.54 (d, 8.9 Hz) and δ_H 4.09 (d, 8.9 Hz)] was observed, corresponding to the oxidation of C-17 appearing at δ_C 72.0 (t) in the ^{13}C NMR spectrum of **2** by HSQC spectroscopic analysis. Moreover, in the ^{13}C NMR spectrum, one carbon resonance appeared in lower field at δ_C111.3 (s), and it was assigned as C-3 by the long-range heteronuclear correlations of H-2, H_2-4, H-5, and H-15/δ_C 111.3 in the HMBC spectrum (Figure 2). Among the 20 carbons, the existence of one carbonyl, one double bond, and the tetracyclic scaffold of the substrate accounted for six degrees of unsaturation, illustrating the presence of an additional ring system in **2**. Considering the chemical shift of C-3 (δ_C 111.3) and C-17 (δ_C72.0) along with the HR-ESI data of **2**, an additional tetrahydrofuran hemiacetal moiety was proposed. The long-range heteronuclear correlations of H_2-17/C-16, C-1, C-2, and C-3 of the HMBC spectrum (Figure 2) further confirmed a tetrahydrofuran hemiacetal moiety composed of C_1−C_{17}−O−C_3-C_2 in **2**. Because C-3 is an oxygenated tertiary carbon, the configuration of C-3 could not be determined by NOE experiments. Fortunately, suitable crystals for X-ray diffraction were obtained when MeOH−H_2O was used as solvent after several attempts. To validate the above deduction and to determine the stereochemistry of **2**, a single-crystal X-ray diffraction pattern (CCDC 1511323) was obtained by anomalous scattering of Cu Kα radiation. An ORTEP drawing with the atom-numbering scheme indicated is shown in Figure 3 and demonstrates the α orientation of 3-OH, i.e., a 3*S* configuration for C-3 based on the absolute configuration of harzianone reported in 2012.[1] Thus, the structure of **2** was determined as 3α-hydroxy-3β,17-epoxyharzianone.

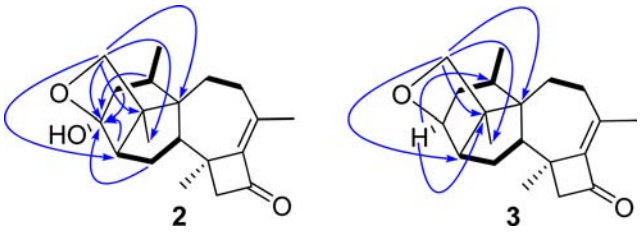

Figure 2. ^1H−^1H COSY (—) and key HMBC (H → C) correlations of **2** and **3**.

The positive HR-ESI-MS spectrum of furanharzianone B (**3**)[10] displayed a quasimolecular ion peak at *m*/*z* 301.2153 $[M + H]^+$ (calcd for $C_{20}H_{29}O_2$, 301.2162), consistent with the molecular formula $C_{20}H_{28}O_2$ and MW 16 amu less than that of **2**, indicating the absence of the 3-OH group in the molecule. The ^1H NMR data of **3** were very similar to those of **2** (Table 1), except for the presence of an oxygenated methine proton signal at δ_H 3.88 (dd, 6.8 Hz, 3.6 Hz). The ^1H−^1H COSY correlations of δ_H 3.88/H-2 and H-4 confirmed the oxygenated methine-3. This was further

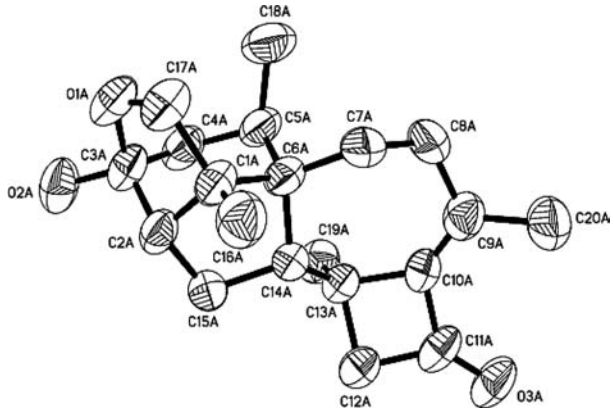

Figure 3. ORTEP diagram of **2**.

supported by the appearance of C-3 at δ_C 74.4 (d) instead of δ_C 111.3 (s) in **2** in the ^{13}C NMR spectrum of **3**. The long-range heteronuclear correlations of H_2-17/C-16, C-1, C-2, and C-6 and H-3/C-1 and C-5 in the HMBC spectrum (Figure 2) further confirmed the above conclusion. The stereochemistry of H-3 was determined to be the α-configuration by NOE experiments, in which the integration value of H-15α was enhanced when H-19 and H-3 were irradiated, respectively (Figure S17). Therefore, the structure of **3** was determined as 3β,17-epoxyharzianone.

By comparison of the spectroscopic data with those reported in the literature, **4** was identified as harziandione[2] (Table S1), a C-3-ketonized product of **1**.

To the best of our knowledge, this is the first report of tetrahydrofuran harziane diterpenoids. Compared with the chemical structures of substrate (**1**) and its metabolites (**2**−**4**) by *Bacillus* sp. IMM-006, a plausible bioconversion route is proposed (Scheme 1). Specific hydroxylation at the C-3 position of **1** yields **1a**, and a subsequent oxidation yields **4**. Compounds **1a** and **4** undergo specific hydroxylation at the C-17 position to form **1b** and **4a**, respectively. As an alternative route, oxidation of **1b** could yield **4a**, from which **2** might be generated by an intramolecular acetalization. In addition, dehydration reaction of 3-OH and 17-OH of **1b** could afford **3**.

Compounds **1**−**4** were evaluated extensively for in vitro cytotoxic[11] (paclitaxel as the positive control), anti-inflammatory[12] (curcumin as the positive control), and anti-HIV[13] (efavirenz as the positive control) activities. The results showed that compounds **1**−**4** displayed no cytotoxic activity at 10^{-5} M, but compounds **1** and **4** exhibited moderate anti-HIV activity with IC_{50} values of 26.1 and 32.6 μM, respectively. Moreover, **1** and **3** exhibited weak anti-inflammatory activity with the inhibition rates of 8.2% and 2.3% at 10^{-6} M as well as 22.5% and 22.7% at 10^{-5} M, respectively.

In summary, this paper reports the structural exploration of harzianone (**1**) by microbial transformation for the first time, and two new compounds (**2** and **3**) were obtained. Compounds **2** and **3** are two unusual 4/7/5/6/5 harziane diterpenoids bearing additional tetrahydrofuran rings composed of C_1−C_{17}−O−C_3−C_2. The reactions observed in the bioconversion might include selective hydroxylation, oxidation of hydroxyl to carbonyl, dehydration between two hydroxyls, and an unusual intramolecular acetalization, which are difficult to access by chemical approach. This work demonstrates that bioconversion with the advantage

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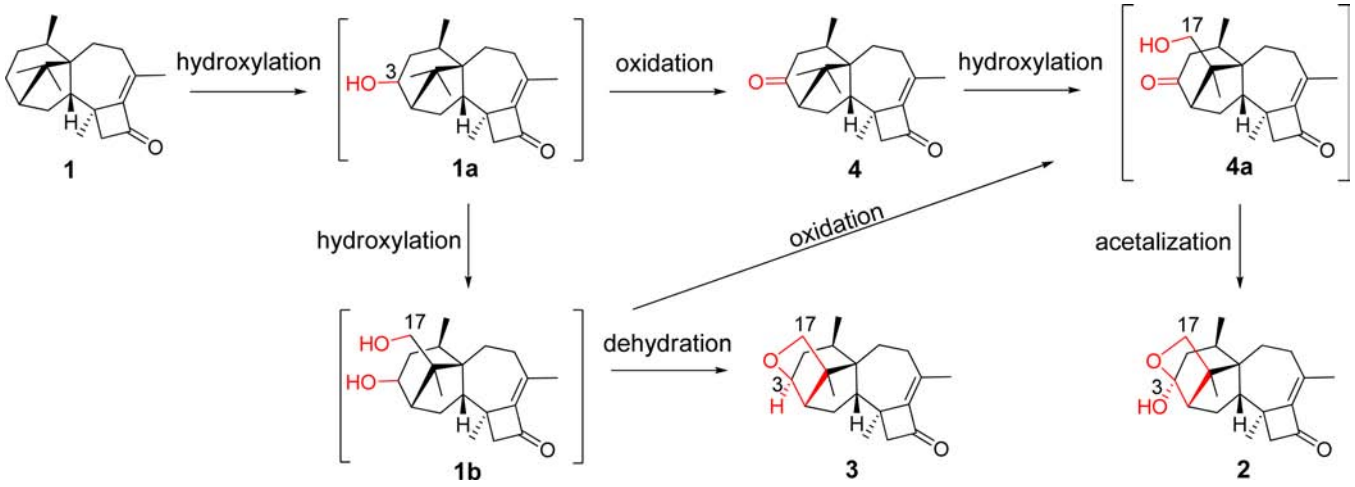
Table 1. ^{1}H and ^{13}C NMR Data of Compounds 2 and 3[a]

	2		3	
no.	δ_C	δ_H[b]	δ_C	δ_H[b]
1	57.8		52.2	
2	54.8	1.97 (1H, m)	47.3	2.00 (1H, m)
3	111.3		74.4	3.88 (1H, dd, 6.8, 3.6)
4	39.8	2.17 (1H, dd, 13.8, 8.6)	35.5	2.50 (1H, m)
		1.55 (1H, m)		1.51 (1H, m)
5	29.9	2.65 (1H, m)	29.3	2.59 (1H, m)
6	53.1		52.8	
7	31.3	1.95 (1H, m)	32.0	2.02 (1H, m)
		1.32 (1H, m)		1.46 (1H, m)
8	30.4	2.45 (1H, m)	30.5	2.46 (1H, m)
		2.02 (1H, ddd, 15.1, 6.5, 1.4)		1.95 (1H, m)
9	148.4		148.9	
10	150.2		150.9	
11	200.5		200.9	
12	60.7	2.63 (1H, d, 16.2)	60.5	2.57 (1H, d, 16.2)
		2.37 (1H, d, 16.2)		2.33 (1H, d, 16.2)
13	41.4		41.5	
14	54.9	2.40 (1H, m)	53.4	2.25 (1H, dd, 11.2, 9.2)
15	24.7	1.86 (1H, ddd, 13.8, 11.0, 7.6, Hβ)	28.0	1.91 (1H, m, Hβ)
		1.59 (1H, m, Hα)		1.16 (1H, dd, 13.6, 9.2, Hα)
16	18.9	1.02 (3H, s)	20.5	1.05 (3H, s)
17	72.0	4.09 (1H, d, 8.9)	67.7	4.42 (1H, d, 11.0)
		3.54 (1H, d, 8.9)		3.54 (1H, d, 11.0)
18	18.5	1.17 (3H, d, 7.5)	22.3	1.21 (3H, d, 7.5)
19	21.4	1.49 (3H, s)	21.4	1.47 (3H, s)
20	22.4	2.08 (3H, s)	22.5	2.07 (3H, s)

[a] ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) in methanol-d_4. [b] Multiplicities and coupling constants (J) in hertz are in parentheses.

Scheme 1. Plausible Bioconversion Routes from 1 to 2−4



of significant regio- and stereoselectivity is an effective means for the unusual modification of compounds with complex structures.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00204.

Experimental procedures; MS, IR, UV, and 1D and 2D NMR for **2** and **3**; MS, 1D NMR data for **1** and **4**; X-ray crystallographic data for **2** (PDF)

X-ray data for **2** (CIF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Miao, F. P.; Liang, X. R.; Yin, X. L.; Wang, G.; Ji, N. Y. *Org. Lett.* **2012**, *14*, 3815−3817.

(2) Ghisalberti, E. L.; Hockless, D. C. R.; Rowland, C.; White, A. H. *J. Nat. Prod.* **1992**, *55*, 1690−1694.

(3) Mannina, L.; Segre, A. L.; Ritieni, A.; Fogliano, V.; Vinale, F.; Randazzo, G.; Maddau, L.; Bottalico, A. *Tetrahedron* **1997**, *53*, 3135−3144.

(4) Adelin, E.; Servy, C.; Martin, M. T.; Arcile, G.; Iorga, B. I.; Retailleau, P.; Bonfill, M.; Ouazzani, J. *Phytochemistry* **2014**, *97*, 55−61.

(5) Xie, Z. L.; Li, H. J.; Wang, L. Y.; Liang, W. L.; Liu, W.; Lan, W. J. *Nat. Prod. Commun.* **2013**, *8*, 67.

(6) Zhang, M.; Liu, J. M.; Zhao, J. L.; Li, N.; Chen, R. D.; Xie, K. B.; Zhang, W. J.; Feng, K. P.; Yan, Z.; Wang, N.; Dai, J. G. *Chin. Chem. Lett.* **2016**, *27*, 957−960.

(7) Betts, R. E.; Walters, D. E.; Rosazza, J. P. *J. Med. Chem.* **1974**, *17*, 599−602.

(8) A two-stage fermentation procedure was used. The cultural medium contained 5 g of yeast extract, 10 g of tryptone, and 10 g of NaCl in 1 L of distilled water. A 1 mL portion of 2-day-old seed cultures of *Bacillus* sp. IMM-006 were added to 40 Erlenmeyer flasks of 250 mL with 100 mL of culture medium for each flask. After 12 h of cultivation, 80.0 mg of harzianone in 1 mL of DMSO was uniformly distributed into the flasks shaken for 4 days, after which the cultures were pooled and centrifuged at 6000*g*. The supernatant was extracted with EtOAc (4 L × 4) and evaporated under reduced pressure to obtain the residue (4.0 g). The residue (4.0 g) was fractionated by silica gel column chromatography (150 g) eluting with petroleum ether−EtOAc (100:0, 100:1, 50:1, 20:1, 10:1, 5:1, 3:1, 1:1, 1:3, v/v) to afford fractions (Fr.) 1−9. Fr. 6 was separated by semipreparative reversed-phase HPLC with MeOH−H_2O (7:3, v/v, 3 mL/min) to afford **4** (4.3 mg, ca. 5.1%; t_R 19.3 min). Fr. 8 was separated by semipreparative reversed-phase HPLC with MeOH−H_2O (11:9, v/v, 3 mL/min) to afford Fr. 8-1 and Fr. 8-2. Fr. 8-1 was further separated by normal-phase semipreparative HPLC using *n*-hexane−EtOAC (1:1, v/v, 4 mL/min) to afford **2** (3.7 mg, ca. 4.2%; t_R 16.2 min) and **3** (1.4 mg, ca. 1.7%; t_R 19.1 min), respectively.

(9) 3*α*-Hydroxy-3*β*,17-epoxy-harzianone (**2**): colorless needle crystals (MeOH−H_2O); $[\alpha]^{20}_D$ +16.0 (*c* 0.13, MeOH); IR (ν_{max}) 3319 (ν_{OH}), 2955, 2878, 1739 ($\nu_{C=O}$), 1666, 1443, 1359, 1259, 1154, and 1026 cm^{-1}; UV (MeOH) λ_{max} (log ε) 254.0 (3.79) nm; HR-ESI-MS *m*/*z* 317.2105 $[M + H]^+$ (calcd 317.2111 for $C_{20}H_{29}O_3$). ^1H and ^{13}C NMR data, see Table 1.

(10) 3*β*,17-Epoxyharzianone (**3**): colorless oil; $[\alpha]^{20}_D$ + 83.3 (*c* 0.06, MeOH); IR (ν_{max}) 2929, 2879, 1734 ($\nu_{C=O}$), 1663, 1451, 1374, 1118, 1023 cm^{-1}; UV (MeOH) λ_{max} (log ε) 255.0 (4.02) nm; HR-ESI-MS *m*/*z* 301.2153 $[M + H]^+$ (calcd 301.2162 for $C_{20}H_{29}O_2$). ^1H and ^{13}C NMR data, see Table 1.

(11) (a) Mosmann, T. J. *J. Immunol. Methods* **1983**, *65*, 55−63. (b) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936−943.

(12) (a) Kim, H. Y.; Park, E. J.; Joe, E.; Jou, I. *J. Immunol.* **2003**, *171*, 6072−6079. (b) Yang, S.; Zhang, D.; Yang, Z.; Hu, X.; Qian, S.; Liu, J.; Wilson, B.; Block, M.; Hong, J. S. *Neurochem. Res.* **2008**, *33*, 2044−2053. (c) Pang, H. Y.; Liu, G.; Liu, G. T. *Acta Pharmacol. Sin.* **2009**, *30*, 209−218.

(13) Zhang, Q.; Liu, Z.; Mi, Z.; Li, X.; Jia, P.; Zhou, J.; Yin, X.; You, X.; Yu, L.; Guo, F.; Ma, J.; Liang, C.; Cen, S. *Antiviral Res.* **2011**, *91*, 321−329.

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