(±)-Terreinlactone A, A Pair of 3-Substituted δ-Lactone Enantiomers Derived from Terrein from the Fungus Aspergillus terreus

Xuwen Zhang\textsuperscript{a,b}, Zhaodi Wu\textsuperscript{a}, Yongji Lai\textsuperscript{c}, Dongyan Li\textsuperscript{d}, Jianping Wang\textsuperscript{a}, Zengwei Luo\textsuperscript{a}, Yongbo Xue\textsuperscript{a}, Hucheng Zhu\textsuperscript{a}, Chunmei Chen\textsuperscript{a,*}, and Yonghui Zhang\textsuperscript{a,*}

\textsuperscript{a} Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
\textsuperscript{b} Humanwell Healthcare (Group) Co., Ltd., Wuhan 430073, China
\textsuperscript{c} Department of Pharmacy, the Central Hospital of Wuhan, Wuhan 430014, China
\textsuperscript{d} Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

* Correspondence e-mail: zhangyh@mails.tjmu.edu.cn (Y. Z.); chenchunmei@hust.edu.cn (C.C.)
Summary

Terreinlactone A (1a/1b), a pair of 3-substituted δ-lactone enantiomers, and terreinlactone B (2), a new biosynthetic intermediate of 1a/1b, were isolated from Aspergillus terreus, along with their biosynthetic precursor (+)-terrein (3) and (+)-isoterrein (4). Compounds 1a and 1b were separated by using a Daicel chiral-pak ASH column eluting with n-hexane–EtOH (80:20). The structures of 1a/1b with absolute configurations were determined by comprehensive spectroscopic analyses and electronic circular dichroic (ECD) calculations. Terreinlactone A (1) represents the first example of 1,5-seco-terrein and a biogenetic pathway is proposed from the precursor terrein via the intermediated terreinlactone B (2).

Keywords: Aspergillus terreus, Terrein, δ-Lactone, Enantiomer, ECD calculation
1. Introduction

*Aspergillus terreus* is a filamentous fungus distribute widely in natural world, and from the culture broth of it, a variety of natural products have been isolated, such as alkaloids,\(^1,2\) meroterpenoids,\(^3,4\) sesterterpenoids,\(^5\) lignans,\(^2,6,7\) and so on. The most famous metabolite from *A. terreus* is lovastatin (named as mevinolin at first), which is an important HMG-CoA reductase inhibitor clinically used.\(^8,9\) Terrein represents another type of notable metabolites of *A. terreus*, which was firstly isolated in 1935 and structural determined in 1955.\(^10-12\) Then, terrein and its analogues attracted considerable attention for their biosynthetic investigations,\(^13-15\) and until 2014, Hertweck and co-workers illustrated the gene cluster responsible for the biosynthesis of terrein.\(^16\) Besides, terrein and analogues also attracted much attention for their total syntheses\(^17-20\) for their versatile bioactivities including inhibitor of plant growth, anti-microbial, anti-proliferative, and anti-oxidative activities.\(^6,21-24\)

In our previous research, asperterpenes A and B, two meroterpenoids with unusual skeletons and BACE1 inhibite activity, were isolated from the fungus *A. terreus* isolating
from the soil collected in the bottom of Yangzi River.\textsuperscript{4} In the further search for bioactive metabolites from \textit{A. terreus}, a chemical investigation of another \textit{A. terreus} was carried out, which was obtained from China Forestry Culture Collection Center (CFCC 81836).

Unexpectedly, a pair of 3-substituted $\delta$-lactone enantiomers, named (±)-terreinlactone A (±1), derived from terrein, were isolated, along with their biosynthetic intermediate terreinlactone B (2) and precursors (+)-terrein (3) and (+)-isoterrein (4).\textsuperscript{25,26}

![Fig. 1. Structures of compounds 1–4.](image)

### 2. Results and discussion

Terreinlactone A (1) was isolated as a colorless oil. Its molecular formula was determined to be C$_8$H$_{12}$O$_3$ by HRESIMS spectrum with an ion peak at $m/z$ 179.0664 [M + Na]$^+$ (calcd for C$_8$H$_{12}$O$_3$Na, 179.0684), suggesting three degrees of unsaturation. The IR spectrum of 1 showed absorption bands at 3410 and 1714 cm$^{-1}$, suggesting the presence of...
a hydroxyl and an ester carbonyl. The $^1$H and $^{13}$C NMR data (Table 1) along with the HSQC spectrum showed signals of an ester carbonyl ($\delta_C$ 173.4), a disubstituted double bond with $trans$-configuration ($\delta_C$ 137.0 and 125.4; $\delta_H$ 5.75, dq, $J = 15.5$, 6.4 Hz and 5.61, dq, $J = 15.5$, 1.5 Hz), an oxygenated quaternary carbon ($\delta_C$ 70.3), three methylenes including an oxygenated one ($\delta_C$ 67.5, 43.8, and 35.2; $\delta_H$ 4.56, ddd, $J = 11.2$, 10.5, 4.2 Hz; 4.37, ddd, $J = 11.2$, 5.6, 3.8 Hz; 2.69, d, $J = 17.3$ Hz; 2.52, dd, $J = 17.3$, 2.2 Hz; 2.04, ddd, $J = 14.2$, 10.5, 5.6 Hz; 1.84, dddd, $J = 14.2$, 4.2, 3.8, 2.2 Hz), and a methyl ($\delta_C$ 17.8; $\delta_H$ 1.73, dd, $J = 6.4$, 1.5 Hz). These data combined with the degrees of unsaturation revealed one ring system in the structure of 1.

The whole structure of 1 was further elucidated by analyses of the $^1$H−$^1$H COSY and HMBC spectra (Fig. 2). Elucidation of the $^1$H−$^1$H COSY spectrum disclosed the spin systems of C(4)H$_2$−C(5)H$_2$ and C(6)H−C(7)H−C(8)H$_3$. In addition, the $\delta$-lactone ring was further established by the HMBC correlations from H-4 and H-5 to C-3, from H-2 to C-1 and C-3, and from H-5 to C-1. And then, the allyl group of C-6−C-7−C-8, which was elucidated by $^1$H−$^1$H COSY above and further confirmed by HMBC interactions from
Me-8 to C-6 and C-7, was located at C-3 as determined by HMBC correlations from H-6 and H-7 to C-3 and from H-2 to C-6. Thus, the structure of 1 was finally determined.

Fig. 2. $^1$H–$^1$H COSY (bold) and the key HMBC (arrow) correlations of 1 and 2.

Table 1. $^1$H and $^{13}$C NMR Data of 1 and 2 in CD$_3$OD.a

| no. | $\delta_H$ (J in Hz) | $\delta_C$ | $\delta_H$ (J in Hz) | $\delta_C$ |
|-----|----------------------|-----------|----------------------|-----------|
| 1   |                      | 173.4     |                      | 165.7     |
| 2a  | 2.69 d (17.3)        | 43.8      | 5.76 s               | 115.7     |
| 2b  | 2.52 dd (17.3, 2.2)  | 70.3      |                      | 153.7     |
| 3   |                      | 35.2      | 2.53 td (6.2, 0.9)   | 24.0      |
| 4a  | 2.04 ddd (14.2, 10.5, 5.6) | 67.5    | 4.38 t (6.2)         | 66.0      |
| 4b  | 1.84 dddd (14.2, 4.2, 3.8, 2.2) | 5.62     |                      | 130.3     |
| 5a  | 4.56 ddd (11.2, 10.5, 4.2) | 125.4    | 6.20 m               | 135.4     |
| 5b  | 4.37 ddd (11.2, 5.6, 3.8) | 17.8     | 1.89 d (4.9)         | 18.9      |

a 400 MHz for $^1$H and 100 MHz for $^{13}$C, $\delta$ in ppm.

We tried to determine the absolute configuration of 1 by ECD as there is an ester carbonyl close to the stereogenic center of C-3. However, the ECD spectrum showed nearly no Cotton effect, suggesting the racemic feature of 1. The optical rotation of 1 is almost zero, which further indicates that 1 is a racemic mixture. Finally, chiral separation of 1a/1b was performed by using a Daicel chiral-pak ASH column eluting with n-hexane–EtOH (80:20) (Fig. 3) after many attempts with various chiral columns and solvent systems. As
expected, 1a and 1b exhibited mirror-like ECD curves (Fig. 4) and totally opposite optical rotations (1a: \([\alpha]_{D}^{20} +2.4;\) 1b: \([\alpha]_{D}^{20} –3.1\)). TDDFT calculations of the theoretical ECD spectra of 1-S and 1-R were then carried out, which assigned the absolute configurations of 1a and 1b as S and R, respectively. Thus, compounds 1a and 1b were elucidated, and named terreinlactones A1 and terreinlactones A2, respectively.

Fig. 3. HPLC profile for the separation of 1a and 1b on a chiral column eluting with n-hexane–EtOH (80:20).

Fig. 4. Comparison of experimental and calculated ECD spectra of 1a and 1b.

Terreinlactone B (2) was also isolated as a colorless oil. The HRESIMS spectra showed an ion peak at \(m/z\) 161.0566, 18 mass units less than 1, indicating a molecular formula of \(\text{C}_8\text{H}_{10}\text{O}_2\). Further comparing the \(^1\text{H}\) and \(^{13}\text{C}\) NMR data (Table 1) of 2 with those of 1
revealed the presence of an additional double bond ($\delta_C$ 115.7 and 153.7; $\delta_H$ 5.76, s), which was located between C-2 and C-3 by HMBC correlations from H-2 to C-1, C-4, and C-6 (Fig. 2). The whole structure of 2 was assigned by analyses of 2D NMR spectra.

The origin of terrein from five acetate units was preliminarily illuminated in the 1960 to 1980s by using classic isotope-labeling studies. Until 2014, Hertweck and co-workers discovered the PKS biosynthetic pathway of terrein and revealed the gene terA as essential one be responsible for the biosynthesis of terrein. ($\pm$)-Terreinlactone A (1a/1b) was proposed to originate from terrein by reduction to obtain the intermediate iii and then followed by a Baeyer–Villiger oxidation to yield the lactone intermediate 2 (Chart 1).

Finally, compound 2 underwent a hydrolysis reaction to form the enantiomer 1a and 1b.

**Chart 1.** Proposed biosynthesis of terreinlactones A ($\pm$1) and B (2). (* labelled carbons, indicating carbon movement in the proposed biosynthetic pathway.)
Compounds 1a, 1b, and 2 were tested for cytotoxic activities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, SW480) by the MTs method, in vitro. However, both compounds showed no obvious cell proliferation up to a concentration of 40 μM.

In summary, terreinlactone A (1) and B (2) represent the first examples of terrein derivatives with an unusual δ-lactone formed by reduction and Baeyer–Villiger oxidation. The enantiomeric property of (±)-terreinlactone A (1a/1b) was tackled by an enantioseparation procedure and their absolute configurations were determined by ECD calculations.

3. Experimental

3.1 General

HRESIMS was conducted in the positive-ion mode on a Thermo Fisher LC-LTQ-Orbitrap XL spectrometer. UV spectra were recorded with a PerkinElmer Lambda 35 spectrophotometer. IR spectra were measured by a Bruker Vertex 70 FT-IR spectrophotometer. Optical rotations were obtained in a 0.7 mL cell on a Rudolph Autopol.
IV automatic polarimeter. ECD spectra were obtained with a JASCO J-810 spectrometer.

NMR spectra were obtained on a Bruker AM-400 spectrometer, and the $^1$H and $^{13}$C NMR chemical shifts were referenced to the solvent peaks for CD$_3$OD at $\delta_H$ 3.31 and $\delta_C$ 49.0 and DMSO-$d_6$ at $\delta_H$ 2.50 and $\delta_C$ 39.5. Semipreparative HPLC was performed using a Dionex HPLC system equipped with an Ultimate 3000 pump, an Ultimate 3000 autosampler injector, and an Ultimate 3000 DAD detector controlled by the Chromeleon software (version 6.80) using a reversed-phase (RP) C18 column (5 $\mu$m, 10×250 mm, Welch Materials, Inc.), and a Ultimate AQ-RP18 column. Column chromatography (CC) was performed on silica gel (100–200 mesh and 200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), Sephadex LH-20 (40–70 $\mu$m, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and octadecylsilyl (ODS, 50 $\mu$m, YMC Co. Ltd., Japan). TLC was performed with RP-C18 F254 plates (Merck, Germany) and silica gel 60 F254 (Yantai Chemical Industry Research Institute).

3.2 Fungal material
The fungus of *Aspergillus terreus* was provided by China Forestry Culture Collection Center (CFCC 81836).

**3.3 Extraction and isolation**

The strain was cultured on potato dextrose agar (PDA) at 28 °C for 5 days to prepare the seed culture, then inoculated into 300 Erlenmeyer flasks (1 L) which were previously sterilized by autoclaving and each containing 200 g rice and 180 mL distilled water. The flasks were incubated at 28 °C for 28 days. After that, the solid culture was extracted with EtOH, and the EtOH was removed under reduced pressure to yield a crude extract. The crude extract was partitioned with EtOAc against water to obtain the EtOAc fraction (650 g).

The EtOAc fraction was separated by chromatography on a silica gel column chromatography (CC, petroleum ether to EtOAc, 50:1–0:1) to furnish seven fractions (Fr. 1–7). Fr. 3 (12.0 g) was further separated by the ODS column (MeOH–H₂O, 30%–100%) to yield eight subfractions (Fr. 3.1–3.8). Fr. 3.1 (720.0 mg, eluted with MeOH–H₂O, 30:70) was chromatographed on a Sephadex LH-20 column (CH₂Cl₂–MeOH, 1:1) to afford three
subfractions (Fr. 3.1.1–3.1.3). Fr. 3.1.1 was subjected to a silica gel CC (CH₂Cl₂–MeOH, 1:0–0:1) to give four fractions (Fr. 3.1.1.1–3.1.1.4). Fr. 3.1.1.2 was purified by semipreparative HPLC (MeCN–H₂O, 51:49) to afford compound 2 (15.4 mg, tᵣ 14.1 min).

Fr. 4 (18.0 g) was subjected to a silica gel CC (CH₂Cl₂–MeOH, 100:1–0:1) to give four fractions (Fr. 4.1–4.4). Fr. 4.1 was separated by the ODS column (MeOH–H₂O, 30%–100%) to yield six subfractions (Fr. 4.1.1–4.1.6). Fr. 4.1.1 (157.0 mg, eluted with MeOH–H₂O, 30:70) was chromatographed on a Sephadex LH-20 column (CH₂Cl₂–MeOH, 1:1) to afford three subfractions (Fr. 4.1.1.1–4.1.1.3). Fr. 4.1.1.2 was purified by semipreparative HPLC (MeOH–H₂O, 28:72) to afford 1 (a mixture of 1a and 1b) (16.6 mg, tᵣ 20.4 min). Chiral resolution of 1 was performed on Daicel chiral-pak ASH column (eluted with n-hexane–EtOH, 80:20, flow rate 1.0 mL/min, column temperature 29°C) to give 1a (8.0 mg, tᵣ 8.3 min) and 1b (7.8 mg, tᵣ 11.4 min).

Fr. 6 (28.6 g) was subjected to a silica gel CC (CH₂Cl₂–MeOH, 100:1–0:1) to give three fractions (Fr. 6.1–6.3). Fr. 6.2 was separated by the ODS column (MeOH–H₂O, 20%–100%) to yield seven subfractions (Fr. 6.2.1–6.2.7). Fr. 6.2.1 (261.6 mg, eluted with MeOH–H₂O,
(CH$_2$Cl$_2$–MeOH, 1:1) to afford three subfractions (Fr. 6.2.1.1–6.2.1.3). Fr. 6.2.1.2 was purified by semipreparative HPLC (MeOH–H$_2$O, 17:83) to afford compounds 3 (20.5 mg, t$_R$ 32.6 min) and 4 (12.7 mg, t$_R$ 36.2 min).

Terreinlactone A (1): Colorless oil, IR $v_{\text{max}}$ = 3410, 1714 cm$^{-1}$; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) = 203 (2.75) nm; for $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) data see Table 1; HRESIMS [M + Na]$^+$ m/z 179.0664 (calcd for C$_8$H$_{12}$O$_3$Na, 179.0684); 1a: $[\alpha]_{20}^D$ +2.4 (c = 3.6, MeOH); ECD (MeOH) $\lambda$ ($\Delta\varepsilon$) 219 (+0.84) nm; 1b: $[\alpha]_{20}^D$ −3.1 (c = 0.7, MeOH); ECD (MeOH) $\lambda$ ($\Delta\varepsilon$) 219 (−0.78) nm

Terreinlactone B (2): Colorless oil, IR $v_{\text{max}}$ = 1717 cm$^{-1}$; UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) = 265 (4.28) nm; for $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) data see Table 1; HRESIMS [M + Na]$^+$ m/z 161.0566 (calcd for C$_8$H$_{10}$O$_2$Na, 161.0578)

3.4 Computational details

The conformations of 1-S and 1-R generated by BALLOON were subjected to semiempirical PM3 quantum mechanical geometry optimizations using the Gaussian 09
Duplicate conformations were identified and removed when the root-mean-square (RMS) distance was less than 0.5 Å for any two geometry-optimized conformations. The remaining conformations were further optimized at the B3LYP/6-31G(d) level in MeOH with the IEFPCM solvation model using Gaussian 09, and the duplicate conformations emerging after these calculations were removed according to the same RMS criteria above. The harmonic vibrational frequencies were calculated to confirm the stability of the final conformers. The electronic circular dichroism (ECD) spectrum were calculated for each conformer using the TDDFT methodology at the B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) level with MeOH as solvent by the IEFPCM solvation model implemented in Gaussian 09 program. The ECD spectra for each conformer were simulated using a Gaussian function with a bandwidth $\sigma$ of 0.4 eV. The spectra were combined after Boltzmann weighting according to their population contributions.

3.5 Cytotoxic Activities Evaluation
Five human cancer cell lines, including HL-60, SMMC-7721, A-549, MCF-7, and SW-480, together with one noncancerous cell line (Beas-2B), were used in the cytotoxic activity assay as described in our previous report 27.

Acknowledgements

This work was financially supported by the Program for Changjiang Scholars of Ministry of Education of the People's Republic of China (No. T2016088); National natural Science Foundation for Distinguished Young Scholars (No. 81725021); Innovative Research Groups of the National Natural Science Foundation of China (81721005); the National Natural Science Foundation of China (Nos. 81502943 and 31600266); the Academic Frontier Youth Team of HUST; the Integrated Innovative Team for Major Human Diseases Program of Tongji Medical College (HUST); We thank the Analytical and Testing Center at Huazhong University of Science and Technology for assistance in testing of ECD, UV and IR analyses

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials
References and Notes

1) Cai S., Du L., Gerea A. L., King J. B., You J., Cichewicz R. H., *Org. Lett.*, 15, 4186–4189 (2013).

2) He F., Bao J., Zhang X. Y., Tu Z. C., Shi Y. M., Qi S. H., *J. Nat. Prod.*, 76, 1182–1186 (2013).

3) Liaw C. C., Yang Y. L., Lin C. K., Lee J. C., Liao W. Y., Shen C. N., Sheu J.H., Wu S. H., *Org. Lett.*, 17, 2330–2333 (2015).

4) Qi C., Bao J., Wang J., Zhu H., Xue Y., Wang X., Li H., Sun W., Gao W., Lai Y., Chen J. G., Zhang Y., *Chem. Sci.*, 7, 6563–6572 (2016).

5) Liu Z., Chen Y., Chen S., Liu Y., Lu Y., Chen D., Lin Y., Huang X., She Z., *Org. Lett.*, 18, 1406–1409 (2016).

6) Liao W.Y., Shen C.N., Lin L.H., Yang Y.L., Han H.Y., Chen J.W., Kuo S.C., Wu S.H., Liaw C.C., *J. Nat. Prod.*, 75, 630–635 (2012).

7) Sun K., Zhu G., Hao J., Wang Y., Zhu W., *Tetrahedron*, 74, 83–87 (2018).
8) Alberts A.W., Chen J., Kuron G., Hunt V., Huff J., Hoffman C., Rothrock J., Lopez M.,
Joshua H., Harris E., Patchett A., Monaghan R., Currie S., Stapley E.,
Albers–Schonberg G., Hensens O., Hirshfield J., Hoogsteen K., Liesch J., Springer J.,
Proc. Natl. Acad. Sci., 77, 3957–3961 (1980).

9) Tobert J. A., Nat. Rev. Drug Discov., 2, 517–526 (2003).

10) Raistrick H., Smith G., Biochem. J., 29, 606–611 (1935).

11) Barton D. H. R., Miller E., J. Chem. Soc., 1028–1029 (1955).

12) Grove J., J. Chem. Soc., 4693–4694 (1954).

13) Birch A. J., Cassera A., Jones A. R., Chem. Commun., 167–168 (1965).

14) Hill R. A., Carter R. H., Staunton J., J. C. S. Chem. Comm., 380–381 (1975).

15) Hill R. A., Carter R. H., Staunton J., J. Chem. Soc., Perkin Trans. 1, 2570–2576 (1981).

16) Zaehle C., Gressler M., Shelest E., Geib E., Hertweck C., Brock M., Chem. Biol., 21, 719–731 (2014).

17) Auerbach J., Weinreb S. M., J. C. S. Chem. Comm., 298–299 (1974).
18) Klunder A. J. H., Bos W., Zwanenburg B., *Tetrahedron Lett.*, 22, 4557–4560 (1981).

19) Kolb H. C., Martin H., Hoffmann R., *Tetrahedron: Asymmetry*, 1, 237–250 (1990).

20) Altenbach H. J., Holzapfel W., *Angew. Chem. Int. Ed.*, 29, 67–68 (1990).

21) Park S. H., Kim D. S., Kim W. G., Ryoo I. J., Lee D. H., Huh C. H., Youn S. W., Yoo I. D., Park K. C., *Cell. Mol. Life Sci.*, 61, 2878–2885 (2014).

22) Phattanawasin P., Pojchanakom K., Sotanaphun U., Piyapolrungrjoj N., Zungsontiporn S., *Nat. Prod. Res.*, 21, 1286–1291 (2007).

23) Arakawa M., Someno T., Kawada M., Ikeda D., *J. Antibiots.*, 61, 442–448 (2008).

24) Demasi M. A., Felicio L., Pacheco A. O., Leite H. G., Lima C., Andrade L. H., *J. Braz. Chem. Soc.*, 21, 299–305 (2010).

25) Wakana D., Hosoe T., Itabashi T., Nozawa K., Okada K., Campos–Takaki G. M., Yaguchi T., Fukushima K., *Mycotoxins*, 56, 3–6 (2006).

26) Trabolsy Z. B. K. A., Anouar E. H., Zakaria N. S. S., Zulkeflee M., Hasan M. H., Zin M. M., Ahmad R., Sultan S., Weber J. F. F., *J. Mol. Struct.*, 1060, 102–110 (2014).
27) Zhu H., Chen C., Yang J., Li X. N., Liu J., Sun B., Huang S. H., Li D., Yao G., Luo Z., Li Y., Zhang J., Xue Y., Zhang Y., *Org. Lett.*, **16**, 6322–6325 (2014).