ORIGINAL ARTICLE

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Rec. Nat. Prod. X:X (2023) XX-XX

records of natural products

A New Sesquiterpenoid and Two Nitro-containing Phenylpropionic Acid Derivatives from the Fungus *Aspergillus terreus* LPFH-1

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(Received November 03, 2022; Revised November 29, 2022; Accepted December 08, 2022)

Abstract: Chemical study of the fungal strain *Aspergillus terreus* LPFH-1 led to the isolation of 8 compounds (**1**–**8**), including a new acyclic sesquiterpenoid (**1**) and two new natural products (**2** and **3**). Their structures were determined by extensive analyses of the spectroscopic data including 1D (^{1}H and ^{13}C NMR) and 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC, NOESY) data. Compounds **2** and **3** were nitro-containing phenylpropionic acid derivatives, whose absolute configuration was determined by comparing their specific rotations with those of synthetic products. The known compounds were identified as 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (**4**), ethyl 2,4-dihydroxy-5,6-dimethylbenzoate (**5**), terretonin D (**6**), asperterpene K (**7**), and asterrelenin (**8**).

Keywords: marine-derived fungus; *Aspergillus terreus* LPFH-1; new sesquiterpenes; two nitro-containing phenylpropionic acid derivatives.

1. Introduction

Marine-derived *Aspergillus* strains have been proved to be prolific to produce metabolites with distinctive structures [1-8]. The species *Aspergillus terreus* was a distinguished member, which produced butenolides [9-13], meroterpenoids [14-16], sesterterpenoids [17], alkaloids [18], and cyclic peptides [18]. Some exhibited significant bioactivities, such as the butenolide derivatives, which have been reported to possess noteworthy α-glucosidase inhibitory [10] and promising antiallergic effects [11].

In our study, the strain *Aspergillus terreus* LPFH-1 was isolated and identified, its HPLC fingerprint was very prolific. Various chromatographic separations of this strain were carried out, leading to the isolation of eight compounds (Figure 1), including a new acyclic sesquiterpene (**1**), two nitro-containing phenylpropionic acid derivatives (**2** and **3**), two benzene derivatives (**4** and **5**), two 3,5-dimethylorsellinic acid-based meroterpenoids (**6** and **7**), and a known alkaloid (**8**). Compounds **2** and **3** were new natural products. The isolation and structural identification of these compounds were described herein.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 202x EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.375.2211.2623
Available online: Jnauary 07, 2023

2. Materials and Methods

2.1. General Experimental Procedures

Specific rotations were measured by an SGW®-1 automatic polarimeter. UV spectra were measured on a Cary 300 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-400FT NMR spectrometer. HRESIMS spectrum was obtained on a Waters Xevo G2 Q-TOF spectrometer fitted with an ESI source. Semi-preparative high-performance liquid chromatography (HPLC) was undertaken on a Shimadzu LC-6AD pump using a UV detector, and a YMC-Pack ODS-A column was used for separation.

2.2. Microorganism Material

The strain LPFH-1 was isolated from sea sediments collected from the Hangzhou Bay, which was identified to be *Aspergillus terreus* by comparison of the ITS region of the rDNA sequence with those recorded in GenBank. The sequence was delivered to the GenBank (http://www.ncbi.nlm.nih.gov) with the accession number OP753575.

2.3. Fermentation and Isolation

The fermentation was conducted in 20 fernbach flasks (500 mL) containing 80 g of rice and 90 mL of distilled water. The contents were soaked for 6 h before autoclaving in a steam sterilizer. The fresh mycelia of the target strain were grown on PDA medium at room temperature (r.t.) for 4 days and were then transferred into the flasks. The mycelia were further incubated at r.t. for 30 days.

The fermented materials were extracted with EtOAc (3 × 4000 mL). After evaporation under vacuum, the extract (1.5 g) was split on an ODS silica gel column chromatography (CC) using MeOH/H_2O (20:80→100:0) as eluent to obtain eight fractions (F1–F8). F5 was separated on a semi-preparative YMC-pack ODS-A column (*S*-5 μm, 12 nm, 250 × 12 mm) using ACN/H_2O (47:53, 3 ml/min) to give four subfractions (F5a–F5d). F5b was purified on Sephadex LH-20 CC (MeOH) to afford **4** (1.6 mg) and **5** (2.1 mg). F6 was further chromatographed over a reversed-phase silica gel CC eluted with MeOH/H_2O (40:60→100:0) to obtain five subfractions F6a–F6e. F6b was separated by HPLC using ACN/H_2O (55:45, 3 mL/min, C18 column) to obtain **2** (5.5 mg). F6c was separated by HPLC using ACN/H_2O (56:44, 3 mL/min, C18 column) to obtain **1** (34.7 mg). F6d was chromatographed by HPLC with ACN/H_2O (60:40, 3 mL/min, C18 column) as eluent to abtain **3** (1.1 mg). F6e was separated by HPLC eluted with ACN/H_2O (60:40, 3 mL/min, C18 column) to get **7** (2.4 mg). F7 was further chromatographed over C-18 silica gel CC eluted with MeOH/H_2O (50:50→100:0) to give F7a–F7g. F7d was separated by HPLC using ACN/H_2O (71:29, 3 mL/min, C18 column) as eluent to afford **6** (4.2 mg) and **8** (2.5 mg).

*Deacetylated aspterric A (**1**)*: Colorless oil; $[\alpha]^{25}_{D}$ 0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.09), 286 (3.15) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 269.1748 $[M - H]^-$ (calcd. for $C_{15}H_{25}O_4$, 269.1758).

*(R)-2-(4-Nitrobenzyl)propanoic acid (**2**)*: Colorless oil; $[\alpha]^{25}_{D}$ −83 (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 208.10 $[M - H]^-$ (calcd. for $C_{10}H_{10}NO_4$, 208.06), 232.08 $[M + Na]^+$ (calcd. for $C_{10}H_{11}NO_4Na^+$, 232.06).

*Methyl α-methyl-4-nitrobenzenepropanoate (**3**)*: Colorless oil; $[\alpha]^{25}_{D}$ −72 (*c* 0.01, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 246.30 $[M + Na]^+$ (calcd. for $C_{11}H_{13}NO_4Na^+$, 246.07).

3. Results and Discussion

3.1. Structure Elucidation

The molecular formula of compound **1** was determined to be $C_{15}H_{26}O_4$ by the HRESIMS, requiring 3 degrees of unsaturation. The ^{1}H NMR and HSQC spectra exhibited resonances for three methyl singlets including two olefinic methyls [δ_H 1.28 (3H, s, H_3-12), 1.63 (3H ,s, H_3-13), 1.65 (3H, s, H_3-15)], two olefinic protons [δ_H 5.15 (1H, t, *J* = 7.0 Hz, H-6), 5.38 (1H, t, *J* = 7.0 Hz, H-10)], and six methylenes [δ_H 1.58 (2H, m, H_2-4), 2.02 (2H, t, *J* = 7.0 Hz, H_2-8), 2.09 (2H, m, H_2-5), 2.14 (2H, q, *J* = 7.0 Hz, H_2-9), 2.45 (2H, s, H_2-2), 3.91 (2H, s, H_2-14)]. The ^{13}C NMR spectrum revealed 15 carbon resonances totally, inclusive of four olefinic carbons (δ_C 125.7, 126.5, 135.9, 135.9) for two double bonds, and a carboxylic acid carbon (δ_C 175.7). The carboxyl carbon and the two double bonds were accounted for all three degrees of unsaturation, indicating **1** to be acyclic. The above-mentioned data were very similar to those of aspterric A with the only distinction owing to the absence of the acetyl group, indicating **1** was the deacetyled derivative of aspterric A [19]. The structure of **1** was secured by detailed interpretation of 2D NMR data (Figure 2). The ^{1}H–^{1}H COSY relationship from H_2-4 (δ_H 2.09) to H-6 (δ_H 5.15) and from H_2-8 (δ_H 1.63) to H-10 (δ_H 5.38) established two proton-bearing fragments, which were further assembled with other structural units via HMBC correlations from the three methyls to the around carbons and from the methylene at δ_H 2.45 to the carboxylic acid group (δ_C 175.7). The Δ^6 and Δ^{10} were established to be *E*-configuration as determined by NOE correlations of H_3-13 (δ_H 1.63)/H_2-5 (δ_H 2.09), H_2-8 (δ_H 2.02)/H-6 (δ_H 5.15), H_3-15 (δ_H 1.64)/H_2-9 (δ_H 2.14), and H_2-14 (δ_H 3.91)/H-10 (δ_H 5.38). The specific rotation of **1** ($[\alpha]^{20}_D$ 0) indicated that **1** was racemic. Hence, the structure of **1** was determined to be deacetylated aspterric A.

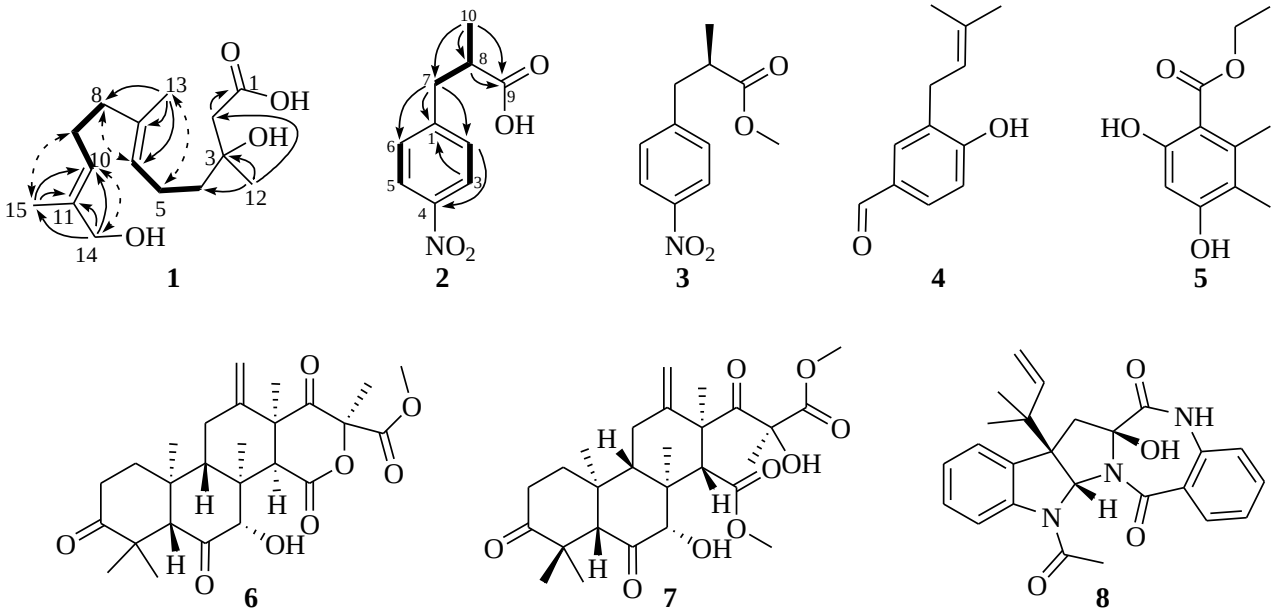

Figure 1. Key ^{1}H–^{1}H COSY (—) and HMBC correlations (⤻) of **1** and **2**, key NOESY correlations (↞⇢) of **1**, and the structures of compounds **1**–**8**

The molecular weight of **2** was determined to be 209.10 by the ESIMS. The ^{1}H NMR spectrum exhibited the presence of a 1,4-disubstituted benzene ring [δ_H 8.15 (2H, d, *J* = 8.7 Hz), 7.46 (2H, d, *J* = 8.7 Hz)], a methyl doublet [δ_H 1.18 (3H, d, *J* = 6.6 Hz)], and three alkyl protons (δ_H 3.09, 2.83, 2.78).

The ^{13}C NMR and HSQC spectra exhibited a total of 10 carbon resonances, including six carbons for a benzene ring and a carbonyl carbon for a carboxyl group (δ_C 179.4), along with a methine (δ_C 42.4) and a methylene (δ_C 40.3). The ^1H–^1H COSY relationship of H-8 (δ_H 2.78)/H_2-7 (δ_H 3.09, 2.83) and H-8/H_3-10 (δ_H 1.18), as well as the HMBC correlations from H-8 and H_3-10 to the carboxyl carbon C-9 (δ_C 179.4) assigned an isobutyric acid unit (Figure 2). The isobutyric acid unit was connected to the benzene ring at C-1 (δ_C 148.0) by HMBC correlations from H_2-8 to C-1 (δ_C 148) and C-2 (δ_C 131.2). A nitro group was positioned at C-4 as determined by the ESIMS data in combination with the chemical shifts of C-4 (δ_C 148.1). Thus, the gross structure of **2** was established to be 2-(4-nitrobenzyl)propanoic acid, the absolute configuration of the only chiral center C-8 was determined to be *R* by comparing its specific (**2**: $[\alpha]^{20}_D$ −68) with that of that of the synthetic product (*R*)-2-(4-nitrobenzyl)propanoic acid [20]. Compound **2** was elucidated to be (*R*)-2-(4-nitrobenzyl)propanoic acid.

The ^1H NMR and ^{13}C NMR data of **3** were very similar to those of **2** except for the presence of a methoxyl group (δ_H 3.61; δ_C 52.2), suggesting **3** to be a methyl ester derivative of **2**. The HMBC correlations from the methoxyl protons to the carbonyl carbon at δ_C 177.5 confirmed the structure. The absolute stereochemistry of **3** was assigned as *R*-configuration by comparing its specific rotation (**3**: $[\alpha]^{20}_D$ −56) with those of the synthetic products in the literature ($[\alpha]^{20}_D$ +45.3 (*c* = 1.0, EtOH) for (*S*)-**3**; $[\alpha]^{20}_D$ −49.1 (*c* = 1.0, EtOH) for (*S*)-14) [21].

Additionally, the rest known compounds were assigned to be 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (**4**) [22], ethyl 2,4-dihydroxy-5,6-dimethylbenzoate (**5**) [23], terretonin D (**6**) [24], asperterpene K (**7**) [25], and asterrelenin (**8**) [24] based on sharing almost identical NMR data with the assigned structures reported in the literature.

Table 1. ^1H and ^{13}C NMR Data of **1–3** in Methanol-d_4. [a]

No.	**1**			**2**		**3**	
	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
1		175.7	1		148.0		148.9
2	2.45, s	46.5	2	7.46, d (8.7)	131.2	7.44, d (8.7)	131.2
3		72.1	3	8.15, d (8.7)	124.4	8.16, d (8.7)	124.5
4	1.58, m	42.7	4		148.0		148.1
5	2.09, m	23.5	5	8.15, d (8.7)	124.4	8.16, d (8.7)	124.5
6	5.15, t (7.0)	125.7	6	7.46, d (8.7)	131.2	7.44, d (8.7)	131.2
7		135.9					
8	2.02, t (7.0)	40.4	7	3.09, m 2.83, m	40.3	3.08, m 2.86, m	40.3
9	2.14, q (7.0)	27.3	8	2.78, m	42.4	2.85, m	42.3
10	5.38, t (7.0)	126.5	9		179.4		177.5
11		135.9	10	1.18, d (6.7)	17.5	1.18, d (6.6)	17.4
12	1.28, s	27.1					
13	1.63, s	16.0				3.61, s	52.2
14	3.91, s	69.0					
15	1.64, s	13.7					

[a] ^1H NMR recorded at 400 MHz, ^{13}C NMR recorded at 100 MHz.

3.2. Cytotoxicity Activity

Compounds **1**–**8** were evaluated for their cytotoxicity toward their inhibitory activity against the human leukemia cell line K562 at an initial concentration of 25 μM by MTT assay [18], while all compounds were inactive with inhibitions less than 30%.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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References

[1] K. W. Wang and P. Ding (2018). New bioactive metabolites from the marine-derived fungi *Aspergillus*, *Mini-Rev. Med. Chem.* **18**, 1072-1094.

[2] C. Zhao, H. Liu and W. Zhu (2016). New natural products from the marine-derived *Aspergillus* fungi-a review, *Acta Microbiol. Sin.* **56**, 331-362.

[3] B. Peng, Q. Peng, J. She, B. Yang and X. Zhou (2022). Secondary metabolites from the coral-derived fungus *Aspergillus terreus* SCSIO41404 with pancreatic lipase inhibitory activities, *Rec. Nat. Prod.* **16**, 639-644.

[4] C. D. Tuan, N. Van Hung, L. T. H. Minh, H. T. H. Lien, J. W. Chae, H. Y. Yun, Y. H. Kim, P. Van Cuong and D. T. M. Huong (2022). A new indole glucoside and other constituents from the sea cucumber-derived *Aspergillus fumigatus* M580 and their biological activities, *Rec. Nat. Prod.* **16**, 633-638.

[5] L. Xu, G. Liu, Y. Chen, S. Liu, W. Luo, P. Hu, C. Huang, X. Ji, S. Wang and G. Cao (2022). Cytotoxic drimane-type sesquiterpenoids from the fungus *Aspergillus flavipes* 297, *Rec. Nat. Prod.*,**16**, 488-492.

[6] R. Yao, S. Yao and A. Hua (2020). Aromatic compounds from the marine-derived fungus *Aspergillus versicolor*, *Rec. Nat. Prod.* **14**, 427-430.

[7] S. T. Fang, X. H. Liu, B. F. Yan, F. P. Miao, X. L. Yin, W. Z. Li and N. Y. Ji (2021). Terpenoids from the marine-derived fungus *Aspergillus* sp. RR-YLW-12, associated with the red alga *Rhodomela confervoides*, *J. Nat. Prod.* **84**, 1763-1771.

[8] C. Sun, X. Liu, N. Sun, X. Zhang, M. Shah, G. Zhang, Q. Che, T. Zhu, J. Li and D. Li (2022). Cytotoxic nitrobenzoyl sesquiterpenoids from an *Antarctica* sponge-derived *Aspergillus insulicola*, *J. Nat. Prod.*, **85**, 987-996.

[9] J. Bao, X. X. Li, F. He, X. Zhang, K. Zhu, H. Tao, J. H. Yu, H. Liu and H. Zhang (2020). Asperbutenolide A, an unusual aromatic butenolide dimer with diverse bioactivities from a marine-derived fungus *Aspergillus terreus* SCAU011, *Tetrahedron Lett.* **61**, 152193.

[10] Z. B. Cheng, Y. L. Li, W. Liu, L. J. Liu, J. Liu, W. J. Yuan, Z. H. Luo, W. Xu and Q. Li (2019). Butenolide derivatives with α-glucosidase inhibitions from the deep-sea-derived fungus *Aspergillus terreus* YPGA10, *Mar. Drugs.* **17**, 332

[11] I. S. Uras, S. S. Ebada, M. Korinek, A. Albohy, B. S. Abdulrazik, Y.-H. Wang, B.-H. Chen, J.-T. Horng, W. Lin, T.-L. Hwang and B. Konuklugil (2021). Anti-inflammatory, antiallergic, and COVID-19 main protease (Mpro) inhibitory activities of butenolides from a marine-derived fungus *Aspergillus terreus*, *Molecules* **26**, 3354.

[12] Q. Peng, W. Chen, X. Lin, J. Xiao, Y. Liu and X. Zhou (2022). Butenolides from the coral-derived fungus *Aspergillius terreus* SCSIO41404, *Mar. Drugs.* **20**, 212.

[13] C. Qi, W. Gao, D. Guan, J. Wang, M. Liu, C. Chen, H. Zhu, Y. Zhou, Y. Lai, Z. Hu, Q. Zhou and Y. Zhang (2018). Butenolides from a marine-derived fungus *Aspergillus terreus* with antitumor activities against pancreatic ductal adenocarcinoma cells, *Bioorg. Med. Chem.* **26**, 5903-5910.

[14] K. Sun, G. Zhu, J. Hao, Y. Wang and W. Zhu (2018). Chemical-epigenetic method to enhance the chemodiversity of the marine algicolous fungus, *Aspergillus terreus* OUCMDZ-2739, *Tetrahedron* **74**, 83-87.

[15] H.-L. Li, X.-M. Li, X. Li, S. Q. Yang and B. G. Wang (2019). Structure, absolute configuration and biological evaluation of polyoxygenated meroterpenoids from the marine algal-derived *Aspergillus terreus* EN-539, *Phytochem. Lett.* **32**, 138-142.

[16] C. J. Wu, X. Cui, B. Xiong, M.-S. Yang, Y. X. Zhang and X. M. Liu (2019). Terretonin D1, a new meroterpenoid from marine-derived *Aspergillus terreus* ML-44, *Nat. Prod. Res.* **33**, 2262-2265.

[17] Z. Liu, Y. Chen, S. Chen, Y. Liu, Y. Lu, D. Chen, Y. Lin, X. Huang and Z. She (2016). Aspterpenacids A and B, two sesterterpenoids from the mangrove endophytic fungus *Aspergillus terreus* H010, *Org. Lett.* **18**, 1406-1409.

[18] F. He, J. Bao, X. Y. Zhang, Z. C. Tu, Y. M. Shi and S. H. Qi (2013). Asperterrestide A, a cytotoxic cyclic tetrapeptide from the marine-derived fungus *Aspergillus terreus* SCSGAF0162, *J. Nat. Prod.* **76**, 1182-1186.

[19] Y. L. Li, W. Liu, W. Xu, X. Zeng, Z. B. Cheng and Q. Li (2020). Aspterrics A and B, new sesquiterpenes from deep sea-derived fungus *Aspergillus terreus* YPGA10, *Rec. Nat. Prod.* **14**, 18-22.

[20] H.-W. Shih, M. N. Vander Wal, R. L. Grange and D. W. C. MacMillan (2010). Enantioselective α-benzylation of aldehydes via photoredox organocatalysis, *J. Am. Chem. Soc.* **132**, 13600-13603.

[21] Q. F. Wu, P. X. Shen, J. He, X. B. Wang, F. Zhang, Q. Shao, R. Y. Zhu, C. Mapelli, J. X. Qiao, M. A. Poss and J. Q. Yu (2017). Formation of α-chiral centers by asymmetric β-C(sp3)-H arylation, alkenylation, and alkynylation, *Science* **355**, 499-503.

[22] M. Vu, L. Herfindal, O. J. Juvik, A. Vedeler, S. Haavik and T. Fossen (2016). Toxic aromatic compounds from fruits of *Narthecium ossifragum* L, *Phytochemistry* **132**, 76-85.

[23] G. H. Silva, H. L. Teles, H. C. Trevisan, V. d. S. Bolzani, M. C. M. Young, L. H. Pfenning, M. N. Eberlin, R. Haddad, C. M. Costa-Neto and A. R. Araujo (2005). New bioactive metabolites produced by *Phomopsis cassiae*, an endophytic fungus in *Cassia spectabilis*, *J. Braz. Chem. Soc.* **16**, 1463-1466.

[24] G. Y. Li, B. G. Li, T. Yang, J. H. Yin, H. Y. Qi, G. Y. Liu and G. L. Zhang (2005). Sesterterpenoids, terretonins A−D, and an alkaloid, *asterrelenin*, from *Aspergillus terreus*, *J. Nat. Prod.* **68**, 1243-1246.

[25] C. Qi, M. Liu, Q. Zhou, W. Gao, C. Chen, Y. Lai, Z. Hu, Y. Xue, J. Zhang, D. Li, X. N. Li, Q. Zhang, J. Wang, H. Zhu and Y. Zhang (2018). BACE1 Inhibitory meroterpenoids from *Aspergillus terreus*, *J. Nat. Prod.* **81**, 1937-1945.