A 2,4′-linked tetrahydroxanthone dimer with protein tyrosine phosphatase 1B inhibitory activity from the Okinawan freshwater Aspergillus sp.

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NOTE

A 2,4′-linked tetrahydroxanthone dimer with protein tyrosine phosphatase 1B inhibitory activity from the Okinawan freshwater *Aspergillus* sp.

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In the course of our research on protein tyrosine phosphatase (PTP) 1B inhibitors, we identified a new 2,4′-linked tetrahydroxanthone dimer (**1**), named secalonic acid F1 (Figure 1a) in the culture broth of the Okinawan freshwater fungus *Aspergillus* sp. TPU1343. This fungus produced the unusual bis-tetrahydroxanthone, asperdichrome.[1] PTP1B plays an important role as a negative regulator in the insulin and leptin signaling pathways. Therefore, a PTP1B inhibitor is anticipated to become a new type of clinical application for Type 2 diabetes mellitus and obesity.[2–5] We herein describe the fermentation, isolation, structural elucidation, and biological activity of compound **1**.

Aspergillus sp. TPU1343, maintained on a PDA plate, was inoculated into a 100 ml Erlenmeyer flask containing 50 ml of seed medium (2.0% glucose (Wako, Osaka, Japan), 0.50% polypeptone (Wako), 0.050% $MgSO_4{\cdot}7H_2O$ (Wako), 0.20% yeast extract (BD, Franklin Lakes, NJ, USA), 0.10% KH_2PO_4 (Wako), and 0.10% agar (Wako) in freshwater and adjusted to pH 6.0 before sterilization).[1] The flask was shaken reciprocally at 25 °C for 3 days to obtain the seed culture, which was then transferred to the production medium (3.0% sucrose (Wako), 3.0% soluble starch (Wako), 1.0% malt extract (BD), 0.30% Ebios (Asahi Food & Healthcare, Tokyo, Japan), 0.50% KH_2PO_4, and 0.050% $MgSO_4{\cdot}7H_2O$ in freshwater and adjusted to pH 6.0 before sterilization) and cultured at 25 °C for 7 days under agitation.

Acetone (20 l) was added to the culture broth (20 l) and filtered. The filtrate was concentrated *in vacuo* to remove acetone and extracted three times with EtOAc. The EtOAc extract was concentrated *in vacuo* to dryness, and the extract (35 g) was suspended in 30% CH_3OH in H_2O and applied on an ODS column (100 g). The ODS column was eluted stepwise with 30, 50, 70, 85 and 100% CH_3OH in H_2O (200 ml each ×2) to separate 10 fractions (Fr. 1–Fr. 10). Fr. 8 (the second 200 ml of the 85% CH_3OH eluate) was concentrated to give a dark black oil (241 mg), which was purified by preparative HPLC (column; inertsil ODS-3 (GL Science, Tokyo, Japan) 10 × 250 mm; mobile phase, 70% CH_3CN containing 0.05% TFA; detection, UV at 254 nm; flow rate, 2.0 ml min^{-1}) to afford 2.4 mg of compound **1** and 13 mg of secalonic acid F (**2**)[1,6] (Figure 1a).

Compounds in the secalonic acid family typically possess a 2,2′-linked bis-tetrahydroxanthone skeleton as secalonic acids A, E and F (**2**) (Figure 1a).[7] Secalonic acid F (**2**) is a heterodimer consisting of two epimeric monomers at C-5/C-5′, and secalonic acids A and E have homodimeric structures with single isomers (Figure 1a).

Secalonic acid F1 (**1**) was obtained as a yellow oil ($[\alpha]_D^{22} = +75.2$, *c* 0.10, $CHCl_3$), and showed UV absorptions at 201, 249 and 337 nm, and IR bands at 3424, 1740, 1616, 1437, 1234 and 1044 cm^{-1}, similar to **2**.[1,6] The molecular formula of **1** was deduced as $C_{32}H_{30}O_{14}$ by HREIMS data (*m/z* 638.1630 $[M]^+$, Δ −0.6 mmu), which was the same as that of **2**. The ^1H and ^{13}C NMR spectra of **1** (in $CDCl_3$) indicated two sets of signals (Table 1). Two proton signals corresponding to 5-H (δ_H 4.15, s) and 5′-H (δ_H 3.85, d (J = 11.1 Hz)) suggested that compound **1** possessed a heterodimeric tetrahydroxanthone skeleton with the same configuration as that of **2**. Marked differences between **1** and **2** were observed in their HMBC spectra. HMBC correlations from H-3 (δ 7.74) to C-4′ (δ 115.5) and from H-3′ (δ 7.49) to C-2 (δ 118.7) were observed in the spectrum of **1**, which indicated the linkage between two monomeric units at the C-2 and C-4′ positions.

To date, penicillixanthones A and B have been reported as the 2,4′-linked homodimers of secalonic acids A and E, respectively, from the culture broth of *Penicillium thomii*[8] and *Setophoma terrestris*[9] (Figure 1a). Penicillixanthone B was also identified as an antibacterial metabolite from the marine-derived *Penicillium* sp.[10] Comparisons of ^1H and ^{13}C data for **1** with the reported values for penicillixanthones revealed that data for partial structure I (Figure 1b) were similar to those for penicillixanthone B, while data for partial structure II (Figure 1b) were identical to those for penicillixanthone A (Table 1). Thus, the structure of **1** including its relative configuration was assigned as shown in Figure 1a.

The absolute configuration of **1** was proposed to be the same as **2** because heterodimers **1** and **2** were obtained from the same culture

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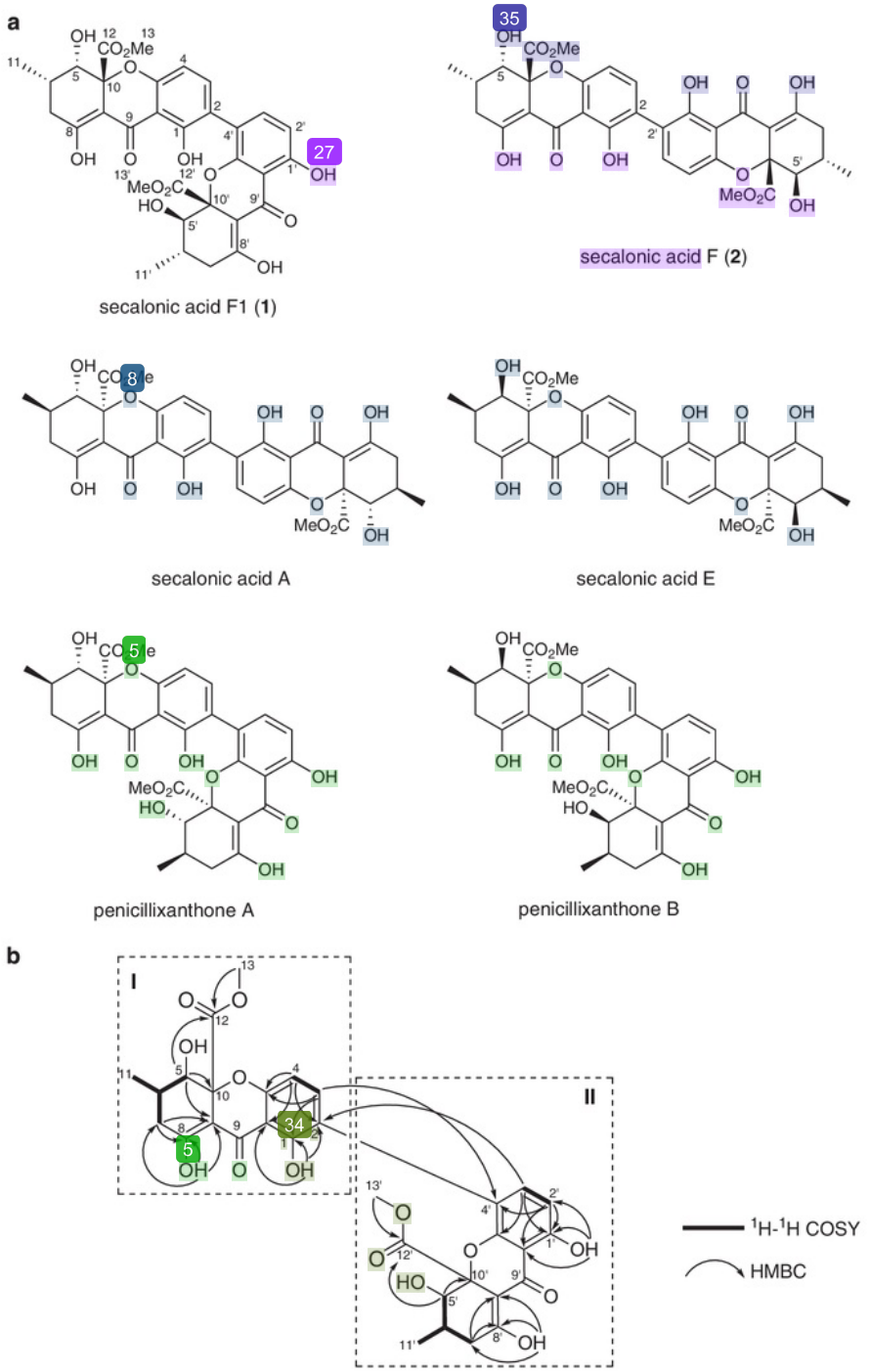

Figure 1 (**a**) Structures of compounds **1** and **2** produced by the freshwater fungus *Aspergillus* sp. TPU1342 and of related compounds. (**b**) ^{1}H–^{1}H COSY and key HMBC correlations for compound **1**.

Table 1 ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for **1** in $CDCl_3$

C#	δ_C	δ_H, mult. (J in Hz)	C#	δ_C	δ_H, mult. (J in Hz)
1	159.4		1′	161.8	
2	118.7		2′	110.5	6.61, d (8.7)
3	139.9	7.74, d (8.7)	3′	140.7	7.49, d (8.7)
4	107.3	6.57, d (8.7)	4′	115.5	
4a	157.1		4′a	155.2	
5	71.3	4.15, s	5′	77.2	3.85, d (11.1)
6	28.6	2.12, m	6′	29.1	2.36, m
7	32.7	(a) 2.41, dd (19.1, 11.3) (b) 2.54, dd (19.1, 11.3)	7′	36.3	(a) 2.27, dd (19.0, 10.5) (b) 2.71, dd (19.0, 6.2)
8	180.0		8′	177.3	
8a	99.9		8′a	101.7	
9	187.6		9′	187.2	
9a	107.1		9′a	107.1	
10	84.9		10′	84.8	
11	17.5	1.19, d (6.5)	11′	17.9	1.11, d (6.5)
12	171.2		12′	170.1	
13	53.5	3.73, s	13′	53.2	3.68, s
1-OH		11.8, s	1′-OH		11.4, s
8-OH		14.0, s	8′-OH		13.7, s

broth of strain TPU1343. This was supported by the positive $n \rightarrow \pi^*$ CD bands of **1** and **2** at 326 nm ($\Delta\varepsilon = +8.6$) and 330 nm ($\Delta\varepsilon = +8.6$), respectively, due to the *R* configurations at the C-10 and C-10′ positions.[1,11,12] Thus, the absolute configuration of **1** was elucidated as (5 *S*, 6 *S*, 10 *R*, 5′ *R*, 6′ *S*, 10′ *R*) (Figure 1a).

Penicillixanthone A (2,4′-linkage, Figure 1a) was previously reported to be transformed from secalonic acid A (2,2′-linkage) in polar solvents such as CH_3CN and pyridine.[13] Qin *et al.*[14] demonstrated that secalonic acid A in DMSO was isomerized to 2,4′- and 4,4′-linked derivatives at room temperature for 10–15 h. Therefore, the transformation of **2** to **1** was examined in CH_3CN, DMSO and CH_3OH. The solution of compound **2** in CH_3CN, DMSO or CH_3OH (1 mg ml^{-1}) was kept at room temperature, and each solution was monitored on 0, 24 and 48 h by HPLC. Compound **2** in CH_3CN was stable for 48 h (Supplementary Figure S1A), and a small peak corresponding to **1** appeared after 24–48 h in DMSO (Supplementary Figure S1B). The isomerization of **2** in DMSO was markedly slower than the reported transformation of secalonic acid A in DMSO.[14] Although CH_3OH was used for ODS column chromatography of the EtOAc extract and to dissolve Fr. 8 for HPLC separation, the conversion of **2** to **1** in CH_3OH was negligible, even after 48 h (Supplementary Figure S1C). During the isolation of compounds **1** and **2**, these compounds were dissolved in CH_3OH only for a few hours. Therefore, it is unlikely that compound **1** was transformed from **2** during the separation procedures. Secalonic acid F1 (**1**) must exist in the fermentation broth of strain TPU1343.

Compound **1** was evaluated for its PTP1B inhibitory activity using the enzyme assay method.[15] PTP1B activity was inhibited by **1** with an IC_{50} value of 5.9 μM (Supplementary Table S1). A positive control, oleanolic acid[16] (Tokyo Chemical Industry, Tokyo, Japan), showed an IC_{50} value of 1.1 μM in the same experiment.

Various cellular functions are controlled by PTPs composed of >100 members including PTP1B,[17] and, thus, selectivity against PTP1B over other PTPs is an important property. The inhibitory activities of **1** against T-cell PTP (TCPTP), one of the non-transmembrane PTPs, CD45 tyrosine phosphatase (CD45), one of the receptor-like PTPs, and *Vaccinia* H-1-related phosphatase (VHR), one of the dual-specificity phosphatases, were examined.[17,18] Compound **1** had IC_{50} values of 6.9 and 6.2 μM against TCPTP and VHR, respectively, similar to PTP1B, while the inhibitory activity of **1** against CD45 was weak ($IC_{50} = 14$ μM) than those against other PTPs (Supplementary Table S1).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Yamazaki, H., Ukai, K. & Namikoshi, M. Asperdichrome, an unusual dimer of tetrahydroxanthone through an ether bond, with protein tyrosine phosphatase 1B inhibitory activity, from the Okinawan freshwater *Aspergillus* sp. TPU1343. *Tetrahedron Lett.* **57**, 732–735 (2016).
2 Qian, S., Zhang, M., He, Y., Wang, W. & Liu, S. Recent advances in the development of protein tyrosine phosphatase 1B inhibitors for Type 2 diabetes. *Future Med. Chem.* **8**, 1239–1258 (2016).
3 Zhang, Z. Y., Dodd, G. T. & Tiganis, T. Protein tyrosine phosphatases in hypothalamic insulin and leptin signaling. *Trends Pharmacol. Sci.* **36**, 66–74 (2015).
4 Wang, L. J., Jiang, B., Wu, N., Wang, S. Y. & Shi, D. Y. Natural and semisynthetic protein tyrosine phosphatase 1B (PTP1B) inhibitors as anti-diabetic agents. *RSC Adv.* **5**, 48822–48834 (2015).
5 Jiang, C. S., Liang, L. F. & Guo, Y. W. Natural products possessing protein tyrosine phosphatase 1B (PTP1B) inhibitory activity found in the last decades. *Acta Pharmacol. Sin.* **33**, 1217–1245 (2012).
6 Andersen, R., Büchi, G., Kobbe, B. & Demain, A. L. Secalonic acids D and F are toxic metabolites of *Aspergillus aculeatus*. *J. Org. Chem.* **42**, 352–353 (1977).
7 Masters, K.-S. & Bräse, S. Xanthones from fungi, lichens, and bacteria: the natural products and their synthesis. *Chem. Rev.* **112**, 3717–3776 (2012).
8 Jiang, T. *et al.* Chemical constituents from marine fungus Penicillium thomii. *Acta Pharm. Sin.* **37**, 271–274 (2002).
9 El-Elimat, T. *et al.* Biosynthetically distinct cytotoxic polyketides from *Setophoma terrestris*. *Eur. J. Org. Chem.* **2015**, 1097–1121 (2015).
10 Bao, J. *et al.* Antifouling and antibacterial polyketides from marine gorgonian coral-associated fungus *Penicillium* sp. SCSGAF 0023. *J. Antibiot.* **66**, 219–223 (2013).
11 Aberhart, D. J., Chen, Y. S., DeMayo, P. & Stothers, J. B. Mould metabolites–IV: the isolation and constitution of some ergot pigments. *Tetrahedron* **21**, 1417–1432 (1965).
12 Franck, B., Gottschalck, E. M., Ohnsorge, U. & Hüper, F. Mutterkorn-Farbstoffe, XII. Trennung, Struktur und absolute Konfiguration der diastereomeren Secalonsäuren A, B und C. *Chem. Ber.* **99**, 3842–3862 (1966).
13 Kurobane, I., Vining, L. C. & McInnes, A. G. (Asahi Kasei Kogyo Kabushiki Kaisha). Secalonic acids. US4424373 (1984).
14 Qin, T., Iwata, T., Ransom, T. T., Beutler, J. A. & Porco, J. A. Jr Syntheses of dimeric tetrahydroxanthones with varied linkages: investigation of "Shapeshifting" properties. *J. Am. Chem. Soc.* **137**, 15225–15233 (2015).
15 Yamazaki, H. *et al.* A polybromodiphenyl ether from an Indonesian marine sponge *Lamellodysidea herbacea* and its chemical derivatives inhibit protein tyrosine phosphatase 1B, an important target for diabetes treatment. *J. Nat. Med.* **67**, 730–735 (2013).
16 Zhang, Y. N. *et al.* Oleanolic acid and its derivatives: new inhibitor of protein tyrosine phosphatase 1B with cellular activities. *Bioorg. Med. Chem.* **16**, 8697–8705 (2008).
17 He, R. J., Yu, Z. H., Zhang, R. Y. & Zhang, Z. Y. Protein tyrosine phosphatases as potential therapeutic targets. *Acta Pharmacol. Sin.* **35**, 1227–1246 (2014).
18 Sumilat, D. A. *et al.* Biphenyl ether derivatives with protein tyrosine phosphatase 1B inhibitory activity from the freshwater fungus *Phoma* sp. *J. Antibiot.* **70**, 331–333 (2017).

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