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Homopetasinic acid isolated from *Diaporthe* sp. strain 1308-05

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ABSTRACT

Homopetasinic acid (**1**) was isolated from fungi of the *Diaporthe* sp. strain 1308-05. NMR spectroscopic structural analysis revealed a petasol (**3**) substructure and a (4*E*,6*E*)-7-carboxy-3-hydroxy-2-methylhepta-4,6-dienoate side chain. The absolute configuration of the petasol moiety was established by the specific rotation value after basic hydrolysis. The (2′*S*,3′*S*)-configuration of the side chain was determined by NMR empirical methods as well as comparison of the spectral data with related model compounds. The absolute structure of the side chain moiety was established on the basis of ECD spectral analyses involving theoretical calculations. The biological activities of **1** are also discussed.

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Introduction

Petasin, an eremophilane-type sesquiterpenoid, was first isolated from roots of *Petasites hybridus* (L.) in 1955.[1] *S*-Petasin is the (*Z*)-3-(methylthio)acrylate derivative found in *P. officinalis*[2] as well as *P. formosanus* Kitamura.[3] The latter has been used as a folk medicine in Taiwan.[4] Since *S*-petasin exhibits remarkable biological properties, such as inhibition of cumulative histamine,[3] spasmolytic activity,[2] and anti-inflammatory activity,[5] it is anticipated to be a novel drug. Although these were isolated from plant resources, we herein report homopetasinic acid (**1**), a 3-*O*-[(4*E*,6*E*,2*S*,3*S*)-3-hydroxy-2-methylocta-4,6-dienedioate] analogue of petasol (**3**), from the filamentous fungus *Diaporthe* sp. strain 1308-05. The structure was elucidated by ESIMS, ^{1}H and ^{13}C NMR, chemical derivatizations, and ECD analyses.

Results and discussion

Isolation and structural elucidation

In the course of exploring novel and bioactive secondary metabolites from fungi in the Shirakami mountainous area Japan, homopetasinic acid (**1**) was isolated along with phomolide C[6] and UCS1025A[7] from the culture broth of *Diaporthe* sp. strain 1308-05. Homopetasinic acid (**1**) afforded a protonated molecular ion at *m*/*z* = 417.2296. The mass spectrum also showed the sodium adduct ion at *m*/*z* = 439.2115, which was helpful to establish the molecular formula of **1** as $C_{24}H_{32}O_6$ ([M+H]$^+$: 417.2277, [M+Na]$^+$: 439.2097). This molecule also gave a dehydrated ion at *m*/*z* = 399.2187, suggesting a hydroxy group ([M−OH]$^+$: 399.2171). The NMR spectral data of **1** in $CDCl_3$ are summarized in Table 1. The ^{1}H-decoupling ^{13}C NMR and HMQC spectra reveal 24 carbon atoms comprising four methyl, four methylene (involving an exomethylene), ten methine, and six quaternary carbons (involving three carbonyls). These analyses also reveal 30 hydrogen atoms directly linked with carbons. The ^{13}C NMR signal at 198.46 ppm is assigned as a ketone, while signals appearing at 169.76 and 174.79 ppm suggest carboxy groups. These were supported by broad but strong absorption found at 1650–1720 cm^{-1} in the IR spectrum. Treatment of **1** with trimethylsilyldiazomethane in methanol gave the monomethyl ester **2** (*m*/*z* = 431.2434), revealing a carboxylic acid functionality in the molecule. Detailed NMR analyses disclosed that **1** comprises a (4*E*,6*E*,2*S*,3*S*)-3-hydroxy-2-methylocta-4,6-dienedioate side chain unit and a petasol substructure. Relative configuration of the petasol moiety was determined by NOEs found at Hβ-1/H-3, Hβ-1/H$_3$-14, H-7/H$_3$-14, and H$_3$-14/H$_3$-15. The (*E*,*E*)-configuration for the C4′,C6′-diene component was established by observing large spin couplings at $^{2}J_{H\text{-}4/H\text{-}5}$ and $^{2}J_{H\text{-}6/H\text{-}7}$ (15.5 Hz for both).

As shown in Figure 1, alkaline hydrolysis of **1** gave an approximate 1:1 mixture of petasol (**3**) and isopetasol (**4**) as well as

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Table 1
NMR spectral data of homopetasinic acid (**1**) in $CDCl_3^*$

Position	$\delta^{13}C$, type	δ^1H (*J* in Hz)	HMBC	NOESY
1	30.49, CH_2	α: 2.36 ddd (2.5, 4.4, 15.1)		Hβ-1, Hα-2, H-9
		β: 2.50 ddt (1.4, 4.9, 15.1)	2, 9, 10	Hα-1, H-3, H_3-14
2	31.47, CH_2	α: 1.48 dddd (4.4, 11.2, 15.1, 16.3)		Hα-1, Hβ-2
		β: 2.14 dddd (2.5, 4.5, 4.9, 16.3)		Hα-2,
3	74.08, CH	4.91 dt (4.5, 11.2)	4, 15, 1′	H_3-14, H_3-15
4	47.06, CH	1.63 dq (11.2, 6.7)	3, 5, 6, 14, 15	Hα-6, H_3-15
5	40.00, C			
6	41.61, CH_2	α: 1.88 t (14.0)	4, 5, 7, 8, 14,	H-4, Hβ-6
		β: 2.03 dd (4.3, 14.0)	5, 7, 8, 10, 14	Hα-6, H-7, H_3-14, H_3-15,
7	50.30, CH	3.12 dd (4.3, 14.0)	6, 8, 11, 12, 13	Hβ-6, H-12, H_3-13, H_3-14
8	198.46, C			
9	124.79, CH	5.8 d (1.4)	1, 6, 7,	Hα-1
10	166.24, C			
11	143.21, C			
12	114.54, CH_2	4.83 brs	7, 13,	H-7
		5.00 brs	7, 13	H_3-13
13	20.05, CH_3	1.74 s	7, 11, 12	
14	17.17, CH_3	1.23. s	4, 5, 6, 10	H-3, Hβ-1, Hβ-6, H-7, H_3-15
15	10.39, CH_3	0.96 d (6.7)	3, 4, 5,	H-3, H-4, Hβ-6, H_3-14
1′	174.79, C			
2′	45.35, CH	2.64 quint (6.7)	1′, 3′, 4′, 9′	H-3′, H-4′, H_3-9′,
3′	73.47, CH	4.37 dt (0.5, 6.7)	1′, 2′, 4′, 5′, 9′	H_3-9′, H-2′, H-4′, H-5′, H-6′
4′	142.62, CH	6.15 dd (6.7, 15.5)	3′, 6′	H-2′, H-3′, H-6′, H_3-9′
5′	129.10, CH	6.48 dd (11.3, 15.5)	3′, 6′, 7′	H-4′, H-7′
6′	145.56, CH	7.32 dd (11.3, 15.5)	4′, 5′, 8′	H-4′, H-5′
7′	120.88, CH	5.93 d (15.5)	5′, 8′	H-5′
8′	169.76, C			
9′	14.13, CH_3	1.25 d (6.7)	1′, 2′, 3′	H-2′, H-3′

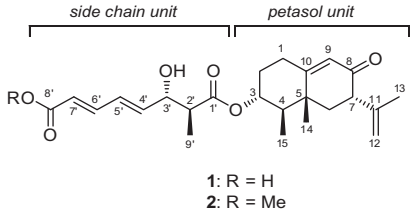
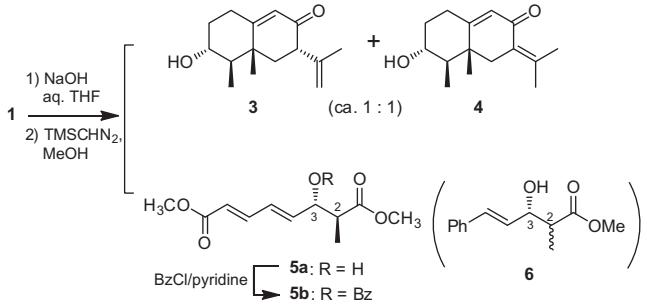


Figure 1. Structures of homopetasinic acid (**1**) and its methyl ester **2**.

(2*E*,4*E*)-6-hydroxy-7-methylocta-2,4-dienedioic acid. The latter was isolated in the form of dimethyl ester **5a** after treatment with trimethylsilyldiazomethane in MeOH. It is known that alkaline hydrolytic conditions accompany the isomerization[8] at the C11-olefin. Although **3** and **4** were not separated, the mixture showed $[\alpha]_D^{25}$ +99 (c 0.52, $CHCl_3$), which is the mean value of pure **3** and **4** from the literature (+130 and +70, respectively).[4,9] This allowed us to conclude the (5*R*)-configuration of **1**.

The configuration of the side chain moiety was determined as follows. The C2, C3 methine carbons, and methyl signal attached to C2 of **5a** appear at 73.53, 45.15, and 14.07 ppm, respectively, in the ^{13}C NMR spectrum in $CDCl_3$. Application of Heathcock's empirical rule suggested a *threo*-configuration between the C2-CH_3 and C3-OH in **5a**.[10] This was further investigated with synthetic model compounds *threo*-**6** and *erythro*-**6**, which were selectively prepared by applying Crimmins's titanium ion mediated Evans's aldol reactions.[11–13] The $^2J_{H\text{-}2/H\text{-}3}$ spin coupling of naturally degraded **5a** is 7.3 Hz in $CDCl_3$ (see Scheme 1), which shows good accordance with the corresponding value of *threo*-**6** (7.2 Hz). In contrast, that of *erythro*-**6** gives an obviously smaller coupling constant (4.2 Hz). The 2-CH_3 of **5a** resembles that of *threo*-**6** (14.22 ppm) rather than *erythro*-**6** (11.45 ppm). These results establish the (2′*S**,3′*S**)-configuration for **1**.

Next, we investigated the absolute configuration of the side chain. The fragment **5a** possesses a dienoate chromophore (λ_{max} = 256 nm, ε = 20,000). Thus, we expected that introduction



	5a	*threo*-6	*erythro*-6
$^2J_{H\text{-}2/H\text{-}3}$ (Hz)	7.3	7.2	4.2
2-CH_3 (ppm)	14.07	14.22	11.45

Scheme 1. Degradation of **1**, chemical transformation of the degradant **5a**, and some characteristic spectral data of **5a** and model compound **6**.

of a benzoyl group (generally, λ_{max} = 230 nm, ε = 15,300) at C3-OH as another chromophore would induce an ECD exciton coupling.[14] Benzoylation of **5a** smoothly proceeded to give **5b** under conventional conditions. As expected, **5b** affords a distinct negatively split ECD curve comprising a negative Cotton effect at 251 nm ($\Delta\varepsilon$ −13) and a positive one at 226 nm ($\Delta\varepsilon$ +15) (Fig. 2). Wavelengths of these Cotton effects agree with the λ_{max} of **5b**. Molecular modeling calculations suggest that stable conformers of the (2*S*,3*S*)-**5b** isomer should show a negative chiral relationship between the chromophores. Theoretical ECD and UV spectra of (2*S*,3*S*)-**5b** show good accordance with the experimental spectra calculated with B3LYP/6-31G*.[6,15–17] These studies establish the entire structure of homopetasinic acid (**1**) shown in Figure 1.

Biological properties

Homopetasinic acid (**1**) inhibited the growth of *Cochliobolus miyabeanus* at 24 μM (IC_{50}). This molecule inhibits the growth of

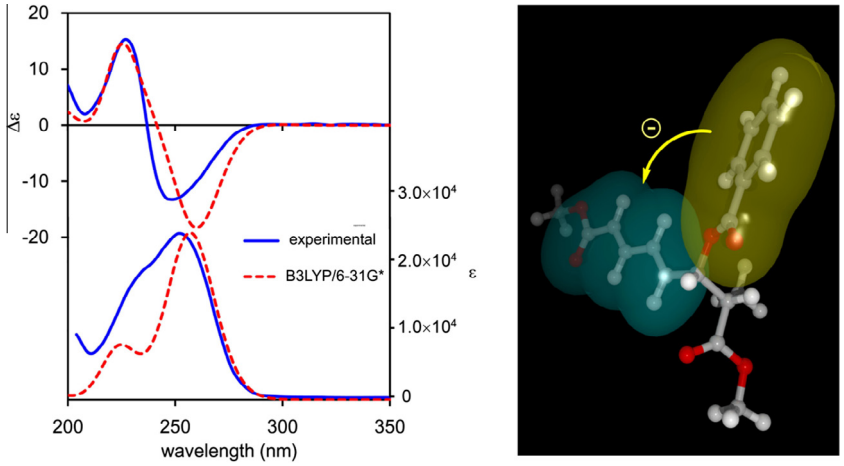


Figure 2. UV and ECD spectra of experimental **5b** (solid line) and theoretical (2*S*,3*S*)-**5b** (dashed line) as well as the 3D structure of its most stable conformation.

human colon adenocarcinoma (COLO 201) cells (IC_{50} ca. 10 μM) based on WST-1 assay.[18] However it was not annihilated at higher concentrations (for example 25 and 100 μM).[19] Further, it exhibits weak inhibition against human promyelocytic leukemia cells HL60 (IC_{50} 105 μM) based on MTT assay.[20] Although the cytotoxicity was comparable to other petasin related penicilleremophilane A [IC_{50} 56.95 (KB cells) or 39.55 μM (MCF-7 cells)], penicilleremophilane B, an analogue without the arylacetate group, has no activities.[21] Additionally, *S*-petasin is known to potently inhibit degranulation of RBL-2H3 cells induced by DNP-BSA (IC_{50} 1.0 nM),[22] **1** showed no activity even in 1×10^3 higher concentration. (IC_{50} > 1000 nM). These results suggest that the structure of the petasin side chains play an important role in the biological activities.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.095.

References and notes

1. Aebi, A.; Buchi, J.; Waaler, T.; Eichenberger, E.; Schmutz, J. *Pharm. Acta Helv.* **1955**, *80*, 277.
2. Aebi, A.; Waaler, T.; Buchi, J. *Pharm. Weekbl.* **1958**, *93*, 397–406.
3. Ko, W.-C.; Lei, C.-B.; Lin, Y.-L.; Chen, C.-F. *Planta Med.* **2001**, *68*, 224–229.
4. Sugama, K.; Hayashi, K.; Nakagawa, T.; Mitsuhashi, H.; Yoshida, N. *Phytochemistry* **1983**, *22*, 1619–1622.
5. Thomet, O. A. R.; Wiesmann, U. N.; Schapowal, A.; Bizer, C.; Simon, H.-U. *Biochem. Pharmacol.* **2001**, *61*, 1041–1047.
6. Ito, A.; Maeda, H.; Tonouchi, A.; Hashimoto, M. *Biosci., Biotechnol., Biochem.* **2015**, *79*, 1067–1069.
7. Nakai, R.; Ogawa, H.; Asai, A.; Ando, K.; Agatsuma, T.; Matsumiya, S.; Akinaga, S.; Yamashita, Y.; Mizukami, T. *J. Antibiot.* **2000**, *53*, 294–296.
8. Herbst, D.; Djerassi, C. *J. Am. Chem. Soc.* **1960**, *82*, 4337–4341.
9. Wang, C.; Li, J.; Yang, R.; Zhao, Y.; Cheng, Y.; Zhang, Z.; He, L. *Rec. Nat. Prod.* **2014**, *8*, 156–164.
10. Heathcock, C. H.; Pirrung, M. C.; Sohn, J. E. *J. Org. Chem.* **1979**, *44*, 4294–4299.
11. Crimmins, M. T.; King, B. W.; Tabet, E. A. *J. Am. Chem. Soc.* **1997**, *119*, 7883–7884.
12. Crimmins, M. T.; McDougall, P. J. *Org. Lett.* **2003**, *5*, 591–594.
13. Crimmins, M. T.; King, B. W.; Tabet, E. A.; Chaudhary, K. *J. Org. Chem.* **2001**, *66*, 894–902.
14. Harada, N.; Nakanishi, K.; Berova, N. In *Comprehensive Chiroptical Spectroscopy: Applications in Stereochemical Analysis of Synthetic Compounds, Natural Products, and Biomolecules*; Berova, N., Polavarapu, P. L., Nakanishi, K., Woody, R. W., Eds.; Wiley: NJ, USA, 2012; pp 115–166.
15. Honmura, Y.; Takekawa, H.; Tanaka, K.; Maeda, H.; Nehira, T.; Hehre, W.; Hashimoto, M. *J. Nat. Prod.* **2015**, *78*, 1505–1510.
16. Arayama, M.; Nehira, T.; Maeda, H.; Tanaka, K.; Miyagawa, H.; Ueno, T.; Hosokawa, S.; Hashimoto, M. *Tetrahedron* **2015**, *71*, 4788–4794.
17. Yasumura, R.; Ashtekar, K. D.; Tonouchi, A.; Nehira, T.; Borhan, B.; Hashimoto, M. *Tetrahedron* **2013**, *69*, 9469–9474.
18. Shirozu, T.; Harada, Y. *Mycoscience* **2004**, *45*, 17–23.
19. That was reproducible. At this moment, we do not have the idea to explain that. Some molecules with cytostatic activity against cancer cells show the similar dose-independent activity.
20. Kimura, K.; Sakamoto, Y.; Fujisawa, N.; Uesugi, S.; Aburai, N.; Kawada, M.; Ohba, S.; Yamori, T.; Tsuchiya, E.; Koshino, H. *Bioorg. Med. Chem.* **2012**, *20*, 3887–3897.
21. Daengrot, C.; Rukachaisirikul, V.; Tansakul, C.; Thongpanchang, T.; Phongpaichit, S.; Bowornwiriyapan, K.; Sakayaroj, J. *J. Nat. Prod.* **2015**, *78*, 615–622.
22. Lee, K.-P.; Kang, S.; Noh, M.-S.; Park, S.-J.; Kim, J.-M.; Chung, H. Y.; Nam, K. J.; Young-Geun, L.; Young-Whan, C.; Dong-Soon, I. *Biomol. Ther. (Seoul)* **2015**, *23*, 45–52.