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Aspewentins A–C, Norditerpenes from a Cryptic Pathway in an Algicolous Strain of *Aspergillus wentii*

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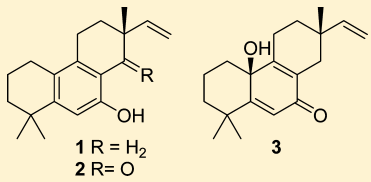
Supporting Information

ABSTRACT: Through addition of suberoylanilide hydroxamic acid, two new aromatic norditerpenes, aspewentins A (**1**) and B (**2**), along with an oxygenated derivative, aspewentin C (**3**), were obtained from the culture of an *Aspergillus wentii* strain (na-3) isolated from the tissue of the brown alga *Sargassum fusiforme*. The structures and absolute configurations were unambiguously elucidated by spectroscopic analyses and quantum chemical calculations. Aspewentins A–C were produced before sporulation and exhibited potent bioactivities against some marine-derived organisms.



1 R = H_2
2 R= O
3

Chemical epigenetic manipulation has been proven to be a reliable method to turn on the silent pathways for secondary metabolites.[1] Following the original work of Cichewicz's group,[2−4] some structurally interesting products were characterized from epigenetically modified fungi.[5,6] Among these studies, histone deacetylase (HDAC) inhibitors, such as suberoylanilide hydroxamic acid (SAHA), have been often used to effectively manipulate the fungal epigenomes.[2,5] The algicolous fungi have been established to be prolific producers of diverse secondary metabolites.[7−11] However, the substrates for culturing them differ greatly from the true habitats, which are difficult to simulate. Thus, media supplemented with SAHA were explored by us. Among the 100 screened fungi, an isolate (na-3) of *Aspergillus wentii* from *Sargassum fusiforme* stood out and exhibited more products than the control. Previously, another *A. wentii* strain also isolated from a *Sargassum* alga was reported to produce tetranorditerpenes.[10] Using an HPLC-monitored separation, three different constituents were isolated herein and identified as new norditerpenes, aspewentins A–C (**1**–**3**). This paper describes the isolation, structure elucidation, and bioactivity of these metabolites.

Compound **1** was obtained as a colorless oil. The molecular formula was determined to be $C_{19}H_{26}O$ by HREIMS, requiring seven degrees of unsaturation. The ^{1}H NMR assignments (Table 1) along with HSQC data showed three methyl singlets, two doublets and one double doublet ascribable to a terminal vinyl group, one singlet attributable to an aromatic proton, and one broad singlet characteristic of an exchangeable proton. A detailed comparison of the data with those reported for 1,4,4,13-tetramethyl-13-vinyl-1,2,3,4,11,12,13,14-octahydrophenanthrene revealed that **1** might be a 1-demethyl-7(or 6)-hydroxy derivative of it.[12] This was supported by the ^{13}C and DEPT NMR spectra (Table 1) with resonances for three methyls, seven methylenes, two methines, and seven nonprotonated carbons. Furthermore, the hydroxy group was assigned to C-7 by the HMBC correlations from the OH to C-6, C-7, and C-8. Other HMBC and COSY correlations validated the planar structure of **1** (Figure 1).

Compound **2** was obtained as a colorless oil. A molecular formula of $C_{19}H_{24}O_2$ was established on the basis of HREIMS data, implying eight degrees of unsaturation. An examination of the ^{1}H, ^{13}C, and DEPT NMR spectral data (Table 1) revealed that **2** was similar to **1** except for the lack of a methylene group and the presence of a carbonyl group in **2**, which was located at C-14 based on HMBC correlations from H-17 to C-12, C-13, C-14, and C-15 and COSY correlations between H-11/H-12 and between H-15/H-16. The downfield-shifted signal for OH-7 indicated the formation of an intramolecular hydrogen bond, which further confirmed the position of the carbonyl group.[9] Thus, the planar structure of **2** was established, which was corroborated by the other HMBC and correlations (Figure 1).

Compound **3** was obtained as a colorless oil. The positive ESIMS spectrum showed a protonated molecule ion peak at m/z 287 $[M + H]^+$ and a sodium adduct ion at m/z 309 $[M + Na]^+$, which suggested a molecular weight of 286. A molecular formula of $C_{19}H_{26}O_2$ was assigned by HRESIMS, indicating seven degrees of unsaturation. It was revealed to be an analogue of compounds **1** and **2** by analysis of the ^{1}H and ^{13}C NMR data (Table 1). A detailed comparison of the data with those reported for yunnannin A and HMBC correlations from H-6 to C-8 and C-10 indicated the presence of a cyclohexadienone ring B,[13] which was fused with ring C through C-8 and C-9 by the HMBC correlations from H-11 and H-14. Additionally, ring A was connected to ring B by the HMBC correlations from H-6 to C-4 and from H-18 and H-19 to C-3, C-4, and C-5; COSY correlations of H-2 with H-1 and H-3 were also noted. Then, C-2 was bonded to C-10 via C-1 to form a six-membered ring based on the elemental composition. The other HMBC and

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Table 1. ^{1}H and ^{13}C NMR Data for 1−3[a]

	1		2		3	
pos.	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1a	26.7, CH_2	2.48, t (6.5)	26.8, CH_2	2.51, m	37.9, CH_2	1.13, m
1b						2.24, m
2a	19.6, CH_2	1.80, m	19.3, CH_2	1.81, m	17.6, CH_2	1.53, m
2b						2.15, m
3a	38.7, CH_2	1.60, m	38.2, CH_2	1.61, m	41.9, CH_2	1.35, m
3b						1.69, m
4	33.8, C		34.3, C		37.5, C	
5	143.8, C		156.2, C		167.7, C	
6	109.9, CH	6.66, s	113.3, CH	6.84, s	122.4, CH	5.99, s
7	151.2, C		160.8, C		185.1, C	
8	119.6, C		114.4, C		128.5, C	
9	135.1, C		142.2, C		157.2, C	
10	126.4, C		124.6, C		70.2, C	
11a	24.2, CH_2	2.55, t (6.6)	23.3, CH_2	2.75, m	21.5, CH_2	2.24, m
11b						2.68, m
12a	34.0, CH_2	1.64, m	34.9, CH_2	2.00, m	33.4, CH_2	1.41, m
12b		1.71, m		2.10, m		1.65, m
13	34.4, C		47.8, C		34.4, C	
14a	34.4, CH_2	2.44, d (16.4)	207.1, C		32.5, CH_2	1.96, brd (17.8)
14b		2.64, d (16.4)				2.44, brd (17.8)
15	147.1, CH	5.89, dd (17.6, 10.8)	140.4, CH	5.98, dd (17.6, 10.8)	145.7, CH	5.76, dd (17.5, 10.8)
16a	111.1, CH_2	4.98, brd (17.6)	115.0, CH_2	5.03, d (17.6)	110.8, CH_2	4.82, brd (17.5)
16b		4.94, brd (10.8)		5.14, d (10.8)		4.91, brd (10.8)
17	25.8, CH_3	1.08, s	23.2, CH_3	1.33, s	27.4, CH_3	1.03, s
18	31.9, CH_3	1.25, s	31.5, CH_3	1.28, s	26.9, CH_3	1.43, s
19	32.0, CH_3	1.25, s	31.6, CH_3	1.27, s	30.4, CH_3	1.16, s
OH		4.44, brs		12.40, s		4.22, brs

[a]Recorded in $CDCl_3$ for **1** and **2** and in acetone-d_6 for **3** at 500 and 125 MHz for ^{1}H and ^{13}C, respectively.

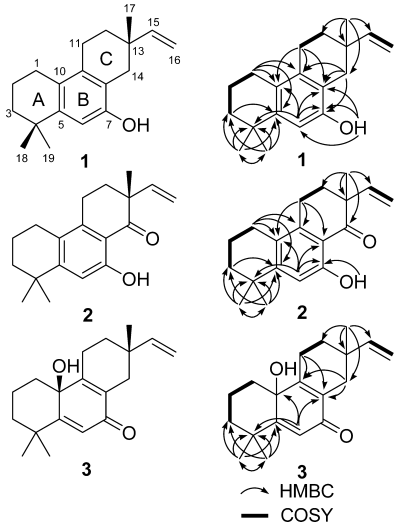

Figure 1. Structures and key HMBC (arrows) and COSY (bold lines) correlations of **1**−**3**.

COSY correlations (Figure 1) further supported the planar structure of **3**, which was deduced to be an oxygenated derivative of **1**.

In order to establish the absolute configurations of compounds **1**−**3**, their electronic circular dichroism (ECD) spectra were determined. However, no Cotton effects were detected in the ECD spectrum of **1** due to the distance between the asymmetric C-13 and the UV chromophore. The ECD spectra of **2** and **3** were computed with the time-dependent density function theory (TD-DFT) method at the gas-phase B3LYP/6-31G(d) level.[14,15] The calculated ECD spectra were produced by SpecDis software,[16] which were in good accordance with the experimental ones (Figures 2 and 3).

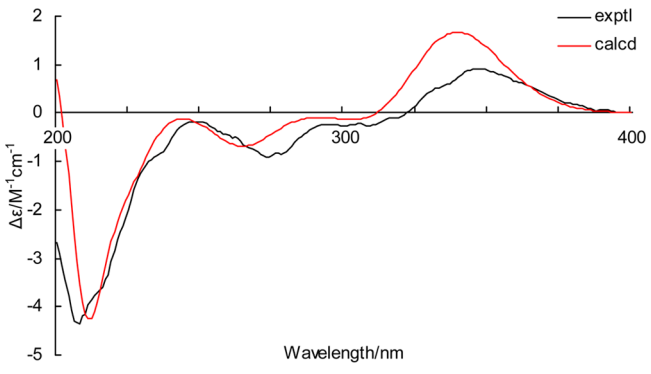

Figure 2. Experimental and calculated ECD spectra of **2**.

Then, the absolute configurations were suggested to be 13*R* for **2** and 10*S* and 13*S* for **3**. On the basis of the biogenic consideration, the absolute configuration of **1** was assigned to be 13*S*. Compounds **1**−**3** were trivially named aspewentins A−C, respectively.

The production of secondary metabolites has been reported to be accompanied by asexual sporulation for some filamentous fungi.[17] However, aspewentins A−C (**1**−**3**) were obtained before the spore formation of *A. wentii* na-3 under the influence

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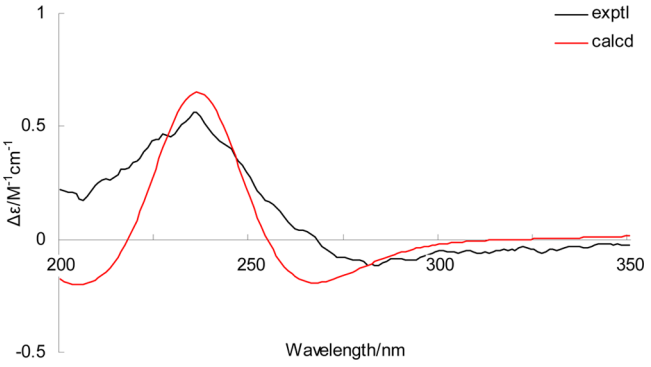

Figure 3. Experimental and calculated ECD spectra of **3**.

of SAHA, which delayed the sporulation of *A. wentii* na-3 relative to the control with plentiful spores at 30 days. Isopimarane diterpenes lacking the CH_3-10 rarely occur in fungi, and this is the first report of aromatic norisopimarane diterpenes from natural sources. It is interesting that some marine algae can produce isopimaranes,[18] which might be further processed by their endophytic fungi with these cryptic pathways.

To evaluate the biological effects of aspewentins A–C (**1**–**3**), they were assayed for growth inhibition against one marine zooplankton (*Artemia salina*) and three marine phytoplankton species (*Chattonella marina*, *Heterosigma akashiwo*, and *Alexandrium* sp.).[8,19] The results (Table S1) showed that **2** was more toxic to *A. salina*, with an LC_{50} of 6.36 μM. Additionally, **1** was more active toward *C. marina* and *H. akashiwo*, with LC_{50} values of 0.81 and 2.88 μM, respectively, and **3** was more active against *Alexandrium* sp., with an LC_{50} of 8.73 μM. Thus, this suggests that the aromatic ring B was critical for some bioactivities of norisopimarane diterpenes.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured on a JASCO P-1020 polarimeter. The UV spectra were measured on a TU-1810 spectrophotometer. The ECD spectra were recorded on a Chirascan CD spectrometer. The IR spectra were obtained on a JASCO FT/IR-4100 spectrometer. The NMR spectra were recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance III 500 NMR spectrometer using TMS as an internal standard. The low- and high-resolution ESI mass spectra were measured on LCQ Fleet and Agilent G6230 TOF mass spectrometers. The low- and high-resolution EI mass spectra were determined on an Autospec Premier P776 mass spectrometer. HPLC separation was carried out on an Agilent HPLC system (1260 infinity quaternary pump, 1260 infinity diode-array detector) using an Eclipse SB-C18 (5 μm, 9.4 × 250 mm) column. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) and Sephadex LH-20 (Pharmacia). TLC was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co.). The solvents were of analytical grade except for the spectral-grade MeOH for HPLC. Quantum chemical calculations were performed using Gaussian 09 software (IA32W-G09RevC.01).

Fungal Material and Fermentation. The fungal strain *A. wentii* na-3 was isolated from the fresh tissue of the surface-sterilized marine brown alga *Sargassum fusiforme* collected from Nanao Island in April 2010. The fungus was identified by morphological observation and analysis of the ITS regions of its rDNA, whose sequence data have been deposited at GenBank with the accession number KF921087. The strain is preserved at the Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences. The initial cultures were maintained on potato dextrose agar plates. Pieces of mycelia were cut into small segments and aseptically inoculated into 30 Erlenmeyer flasks (1 L), each containing 300 mL of Jerusalem artichoke (*Helianthus tuberosus*)/dextrose broth culture media. The media were prepared as follows: Jerusalem artichoke broth, 500 mL/L; dextrose, 10 g/L; $NaNO_3$, 3 g/L; SAHA, 20 μmol/L; natural seawater from the coast of Yantai, 500 mL/L. Static fermentations were performed at room temperature for 30 days.

Extraction and Isolation. The whole cultures (300 mL × 30 flasks, 30 days) were filtered through cheesecloth to separate mycelia from broth. The dried mycelia were homogenized and extracted with a mixture of $CHCl_3$ and MeOH (1:1, v/v), and then the evaporated extract was partitioned between EtOAc and H_2O to yield an EtOAc-soluble extract (5.72 g). Then, it was subjected to silica gel column chromatography (CC) with a step-gradient solvent system consisting of 0–100% petroleum ether (PE)/EtOAc to afford 10 fractions (Frs. 1–10) based on HPLC and TLC analyses. Fr. 3 eluted with PE/EtOAc (20:1) and was further purified by CC on silica gel (PE/EtOAc, 50:1) and Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give compound **1** (6.0 mg). Fr. 7 eluted with PE/EtOAc (10:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel (PE/EtOAc, 30:1) as well as preparative TLC (PE/EtOAc, 10:1) to produce compound **2** (2.2 mg). Fr. 8 eluted with PE/EtOAc (10:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and semipreparative HPLC (MeOH/H_2O, 70–90%) to yield compound **3** (1.4 mg).

*Aspewentin A (**1**):* colorless oil; $[\alpha]^{14}_D$ +41.8 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.77) nm; IR (KBr) ν_{max} 3406, 2924, 1635, 1423, 1080 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (%) 270 (81), 255 (100), 202 (65), 187 (57), 83 (57); HREIMS *m*/*z* 270.1988 [M]$^+$ (calcd for $C_{19}H_{26}O$, 270.1984).

*Aspewentin B (**2**):* colorless oil; $[\alpha]^{20}_D$ +23.3 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 272 (3.93), 347 (3.35) nm; CD (0.42 mg/mL, MeOH), λ_{max} ($\Delta\varepsilon$) 208 (−4.36), 274 (−0.91), 347 (0.91) nm; IR (KBr) ν_{max} 3452, 2927, 2858, 1720, 1635, 1427, 1362, 1080 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (%) 284 (12), 269 (5), 216 (13), 85 (40), 71 (59), 57 (100); HREIMS *m*/*z* 284.1781 [M]$^+$ (calcd for $C_{19}H_{24}O_2$, 284.1776).

*Aspewentin C (**3**):* colorless oil; $[\alpha]^{21}_D$ +8.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 242 (3.28) nm; CD (1.00 mg/mL, MeOH), λ_{max} ($\Delta\varepsilon$) 235 (0.56), 283 (−0.11) nm; IR (KBr) ν_{max} 3429, 2924, 2858, 1709, 1631, 1431, 1377, 1068 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESI$^+$MS *m*/*z* 287 [M + H]$^+$, 309 [M + Na]$^+$; ESI$^-$MS *m*/*z* 285 [M − H]$^-$; HRESIMS *m*/*z* 309.1824 [M + Na]$^+$ (calcd for $C_{19}H_{26}O_2Na$, 309.1830).

Computational Details. Conformational searches for **2** and **3** were performed via the Dreiding force field in MarvinSketch (optimization limit = normal, diversity limit = 0.1) regardless of rotations of methyl and hydroxy groups, the geometries of which were further optimized at the gas-phase B3LYP/6-31G(d) level via Gaussian 09 software to give the energy-minimized conformers without vibrational imaginary frequencies, respectively. Then, the optimized conformers were subjected to the calculations of ECD spectra using TD-DFT at the gas-phase B3LYP/6-31G(d) level, which were drawn via SpecDic software with sigma = 0.25 and 0.35, respectively.

ASSOCIATED CONTENT

Supporting Information

HPLC profiles; NMR, MS, and IR spectra of **1**–**3**; and Cartesian coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Cichewicz, R. H. *Nat. Prod. Rep.* **2010**, *27*, 11−22.
(2) Williams, R. B.; Henrikson, J. C.; Hoover, A. R.; Lee, A. E.; Cichewicz, R. H. *Org. Biomol. Chem.* **2008**, *6*, 1895−1897.
(3) Henrikson, J. C.; Hoover, A. R.; Joyner, P. M.; Cichewicz, R. H. *Org. Biomol. Chem.* **2009**, *7*, 435−438.
(4) Wang, X.; Filho, J. G. S.; Hoover, A. R.; King, J. B.; Ellis, T. K.; Powell, D. R.; Cichewicz, R. H. *J. Nat. Prod.* **2010**, *73*, 942−948.
(5) Vervoort, H. C.; Drašković, M.; Crew, P. *Org. Lett.* **2011**, *13*, 410−413.
(6) Asai, T.; Morita, S.; Shirata, N.; Taniguchi, T.; Monde, K.; Sakurai, H.; Ozeki, T.; Oshima, Y. *Org. Lett.* **2012**, *14*, 5456−5459.
(7) Ji, N. Y.; Liu, X. H.; Miao, F. P.; Qiao, M. F. *Org. Lett.* **2013**, *15*, 2327−2329.
(8) Miao, F. P.; Liang, X. R.; Yin, X. L.; Wang, G.; Ji, N. Y. *Org. Lett.* **2012**, *14*, 3815−3817.
(9) Sun, R. R.; Miao, F. P.; Zhang, J.; Wang, G.; Yin, X. L.; Ji, N. Y. *Magn. Reson. Chem.* **2013**, *51*, 65−68.
(10) Sun, H. F.; Li, X. M.; Meng, L.; Cui, C. M.; Gao, S. S.; Li, C. S.; Huang, C. G.; Wang, B. G. *J. Nat. Prod.* **2012**, *75*, 148−152.
(11) Wang, S.; Li, X. M.; Teuscher, F.; Li, D. L.; Diesel, A.; Ebel, R.; Proksch, P.; Wang, B. G. *J. Nat. Prod.* **2006**, *69*, 1622−1625.
(12) Prawat, U.; Tuntiwachwuttikul, P.; Taylor, W. C.; Engelhardt, L. M.; Skelton, B. W.; White, A. H. *Phytochemistry* **1993**, *32*, 991−997.
(13) Xu, G.; Peng, L. Y.; Lu, L.; Weng, Z. Y.; Zhao, Y.; Li, X. L.; Zhao, Q. S.; Sun, H. D. *Planta Med.* **2006**, *72*, 84−86.
(14) Calculator Plugins were used for structure property prediction and calculation, Marvin 5.9.2, 2012, ChemAxon (http://www.chemaxon.com).
(15) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.
(16) Bruhn, T.; Hemberger, Y.; Schaumlöffel, A.; Bringmann, G. *SpecDis*, Version 1.51; University of Wuerzburg, Germany, 2011.
(17) Calvo, A. M.; Wilson, R. A.; Bok, J. W.; Keller, N. P. *Microbiol. Mol. Biol. Rev.* **2002**, *33*, 447−459.
(18) González, A. G.; Ciccio, J. F.; Rivera, A. P.; Martin, J. D. *J. Org. Chem.* **1985**, *50*, 1261−1264.
(19) Schrader, K. K.; de Regt, M. Q.; Tucker, C. S.; Duke, S. O. *Weed Technol.* **1997**, *11*, 767−774.