Records of Natural Products

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SHORT REPORT

Asperpolyols A and B, two novel hydroxy polyunsaturated fatty acids, isolated from the marine-derived fungus *Aspergillus versicolor* M7

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Abstract: Asperpolyols A ((4*E*,8*E*,12*E*,16*E*)-3,7,11,15-tetrahydroxy-4,6,8,10,12,14,16-heptamethyloctadeca-4,8,12,16-tetraenoic acid) and B ((4*E*,8*E*,12*E*,16*E*)-3,7,11,15-tetrahydroxy-4,6,8,10,12,14,16-heptamethyloctadeca-4,8,12,16-tetraenoic acid), two new hydroxy polyunsaturated fatty acids, were acquired from the extract of *Aspergillus versicolor* M7 isolated from seawater. Their chemical structures were determined by interpretation of 1D/2D nuclear magnetic resonance (NMR) and high-resolution electrospray ionization mass spectrometry (HRESIMS) data. Compounds **1** and **2** were evaluated for inhibiting the growth of three marine phytoplankton species, including *Prorocentrum donghaiense*, *Heterosigma akashiwo*, and *Prorocentrum micans*, and compound **1** exhibited weak inhibitory activity with the half-maximal inhibitory concentration (IC_{50}) values ranging from 62.8 to 86.9 μg/mL.

Keywords: Hydroxy polyunsaturated fatty acids, *Aspergillus versicolor*, secondary metabolites, antimicroalgal activity

1 Fungal Source

A strain of *Aspergillus versicolor* M7 was isolated from seawater samples collected in Yantai in October 2018. The fungal strain was identified as *Aspergillus versicolor* based on the analysis of the internal transcribed spacer (ITS) regions of its ribosomal DNA (rDNA). BLAST search revealed 100% sequence identity with reference strain *Aspergillus versicolor* (GenBank accession number PX360344.1). The corresponding ITS sequence has been deposited in GenBank under the accession number PZ321507. Additionally, the fungal strain was preserved in the School of Life Sciences, Ludong University, located in Yantai, China.

2 Previous Studies

Natural Products (NPs) derived from traditional Chinese medicines, microorganisms, marine organisms and other sources exhibit unique advantages in modern pharmaceutical research due to their structural diversity, multi-target regulatory potential and favorable biological activities (Rodrigues, 2025; Singh et al., 2025; Ali et al., 2025). Extreme marine environments, such as high or low temperature, high hydrostatic pressure, low pH and high salinity, are considered to endow marine secondary metabolites with novel chemical architectures and distinct physiological characteristics. Marine microorganisms play a vital role in the field of natural products owing to their unique biosynthetic pathways (Carroll et al., 2025; Banday et al., 2024). Especially, natural products derived from marine *Aspergillus* species exhibit considerable potential for application in drug discovery and development. To date, no fewer than 300 novel natural products have been reported to be derived from marine *Aspergillus* strains (Wang et al., 2025b). These natural products, with diverse chemical structures and versatile biological activities, have attracted extensive attention from researchers in the fields of natural product chemistry and pharmaceutical science (Wang et al., 2025b; Wang et al., 2025a; Li et al., 2023; Orfali et al., 2021). In addition, the breakout of marine harmful algal blooms (HABs) triggered by toxic microalgae severely disrupts coastal marine ecosystems, produces phycotoxins, and causes heavy losses in aquaculture. Conventional chemical algicides often bring secondary pollution and harm to non-target marine organisms. There is an urgent need to discover eco-friendly algal inhibitors for the control of HABs.

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3 Present Study

As part of our ongoing efforts to discover novel bioactive secondary metabolites from the genus *Aspergillus*, two new hydroxy polyunsaturated fatty acids, designated asperpolyols A and B, were isolated from the organic extract of the marine-derived fungus *Aspergillus versicolor* M7. In this report, the isolation, structural elucidation, and antimicroalgal activity of these two compounds are described.

Static mass fermentation was carried out at 25°C in liquid medium (30 L) consisting of 3% glucose, 0.5% yeast extract powder, and 0.5% peptone dissolved in sterile water. Following 30 days of fermentation, ethyl acetate (EtOAc) was added to the flasks to inactivate the mycelia. The resulting culture broth was extracted four times with EtOAc, and the combined organic phases were concentrated under reduced pressure to obtain a crude extract (42.1 g). Subsequently, the crude extract was subjected to silica gel column chromatography (CC) using step-gradient elution systems of petroleum ether (PE)–EtOAc and dichloromethane (CH_2Cl_2)–methanol (MeOH) (ranging from 1:0 to 0:1, v/v), which afforded 12 fractions. Fraction 7 (4.9 g), eluted with PE–EtOAc (1:1, v/v), was further purified by reversed-phase C18 (RP-18) column chromatography (eluted with MeOH–H_2O, 1:1, v/v) and preparative thin-layer chromatography (TLC), resulting in the isolation of compounds **1** (5.2 mg) and **2** (1.3 mg).

*Asperpolyol A (**1**):* Colorless oil; $[\alpha]_D^{20}$ = +14.3 (*c* = 0.1, MeOH); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 437.2918 $[M - H]^-$ (calcd for $C_{25}H_{41}O_6$, 437.2903).

*Asperpolyol B (**2**):* Colorless oil; $[\alpha]_D^{20}$ = +7.5 (*c* = 0.1, MeOH); ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HRESIMS: *m/z* 369.2303 $[M - H]^-$ (calcd for $C_{20}H_{33}O_6$, 369.2277).

Antimicroalgal activity assay: Antimicroalgal assays were performed against three marine phytoplankton species, including *Heterosigma akashiwo*, *Prorocentrum donghaiense*, and *Prorocentrum micans*. Briefly, each microalgal strain was pre-cultured in artificial seawater medium for 5 days under controlled conditions (20°C, 14:10 h light-dark cycle, 2000 lx light) to reach the exponential growth phase. The cell suspension was then diluted to a working concentration of 4–5 × 10^4 cells/mL, and 195 μL of the diluted culture was dispensed into each well of a 96-well flat-bottom microtiter plate. Subsequently, 5 μL of the test sample solution (prepared in DMSO) was added to each well to achieve final concentrations ranging from 128 to 0.125 μg/mL, followed by thorough mixing. After 24 h of incubation under the same culture conditions, the number of viable algal cells was counted using a hemocytometer under a microscope, and the inhibition rate (IR) was calculated using the following formula: Inhibition rate (IR) = $(N_{CK} - N_T)/N_{CK} \times 100\%$, where N_{CK} represents the number of viable algal cells in the negative control group, and N_T denotes the cell count in the treatment group. The IC_{50} values were calculated by probit analysis using SPSS Statistics. DMSO (2.5%, V/V) and $CuSO_4$ were included as negative and positive controls, respectively, and three replicates were carried out.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **1** and **2** (δ in ppm) in DMSO-d_6

Pos.	1		2	
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	173.1, C		177.5, C	
2	41.5, CH_2	2.25, d (6.6)	43.2, CH	2.17, m
3	73.0, CH	4.19, t (6.7)	79.7, CH	3.75, d (9.2)
4	136.5, C		135.3, C	
5	128.9, CH	5.23, d (9.2)	131.2, CH	5.17, d (9.0)
6	35.8, CH	2.39, m	35.8, CH	2.44, m
7	81.2, CH	3.54, d (7.6)	80.9, CH	3.56, d (7.8)
8	136.1, C		136.1, C	
9	130.7, CH	5.15, d (8.8)	130.11, CH	5.15, d (9.0)
10	35.8, CH	2.42, m	35.8, CH	2.41, m
11	81.1, CH	3.53, d (7.6)	80.6, CH	2.71, d (8.3)
12	136.1, C		62.2, C	
13	130.4, CH	5.17, d (8.3)	56.6, CH	2.84, q (5.4)
14	35.6, CH	2.45, m	13.3, CH_3	1.20, d (5.5)
15	80.9, CH	3.56, d (7.1)	14.6, CH_3	0.79, d (6.9)
16	137.5, C		10.9, CH_3	1.50, s
17	119.8, CH	5.33, q (6.5)	17.6, CH_3	0.71, d (6.8)
18	12.9, CH_3	1.55, d (6.9)	11.6, CH_3	1.55, s
19	11.9, CH_3	1.53, s	17.3, CH_3	0.80, d (6.8)
20	17.5, CH_3	0.68, d (6.5)	11.2, CH_3	1.15, s
21	11.5, CH_3	1.53, s		
22	17.6, CH_3	0.69, d (6.4)		
23	11.6, CH_3	1.53, s		
24	17.7, CH_3	0.71, d (6.7)		
25	11.1, CH_3	1.52, s		

Compound **1** was isolated as a colorless oil. The molecular formula of this compound was determined to be $C_{25}H_{42}O_6$ based on HRESIMS data, which exhibited a deprotonated molecular ion peak at *m/z* 437.2918 $[M - H]^-$ (calcd 437.2903 for $C_{25}H_{41}O_6$). The ^{1}H NMR spectroscopic data (Table 1) showed characteristic proton signals, including four olefinic protons at δ 5.23 (d, *J* = 9.2 Hz, H-5), 5.15 (d, *J* = 8.8 Hz, H-9), 5.17 (d, *J* = 8.3 Hz, H-13), and 5.33 (q, *J* = 6.5 Hz, H-17). Additionally, four oxygen-bearing methine protons were observed at δ 4.19 (t, *J* = 6.7 Hz, H-3), 3.54 (d, *J* = 7.6 Hz, H-7), 3.53 (d, *J* = 7.6 Hz, H-11), and 3.56 (d, *J* = 7.1 Hz, H-15). Moreover, four singlet methyl protons at δ 1.53 (H-19), 1.53 (H-21), 1.53 (H-23), and 1.52 (H-25), as well as four doublet methyl protons at δ 1.55 (d, *J* = 6.9 Hz, H-18), 0.68 (d, *J* = 6.5 Hz, H-20), 0.69 (d, *J* = 6.4 Hz, H-22), and 0.71 (d, *J* = 6.7 Hz, H-24), were unambiguously assigned. The ^{13}C NMR and DEPT spectra of compound **1** displayed 25 carbon resonances, which were categorized into eight methyl carbons, one methylene carbon, eleven methine carbons, and five quaternary carbons. A detailed comparison of the ^{1}H and ^{13}C NMR data revealed that compound **1** shared high structural similarity with the known compound pestalpolyol B (Li et al., 2015). The key structural distinction between the two compounds was identified as the substitution of the butan-2-one group in

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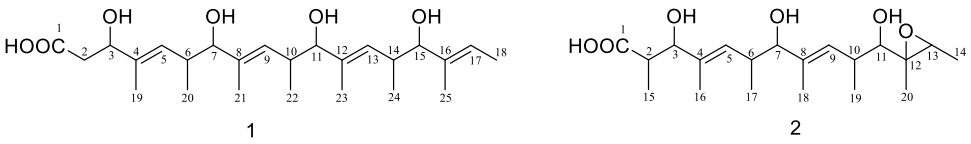


Figure 1. Chemical structures of compounds **1** and **2** isolated from *Aspergillus versicolor* M7

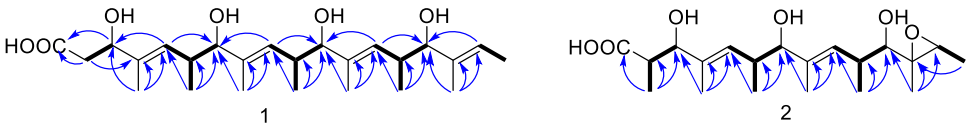


Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of **1** and **2**

pestalpolyol B with a 3-hydroxybutanoic acid moiety in compound **1**. The presence of the 3-hydroxybutanoic acid fragment was further validated by key NMR correlations: the HMBC signals from H-2 and H-3 to C-1, together with the ^{1}H-^{1}H COSY cross-peak between H-2 and H-3, confirmed the skeleton of 3-hydroxybutanoic acid. Furthermore, the HMBC correlations from H-2 to C-4 and from H-19 to C-3, C-4, and C-5 unambiguously located the 3-hydroxybutanoic acid moiety at the C-4 position of the parent skeleton. In addition, the consistent HMBC and ^{1}H-^{1}H COSY correlations of the structural segment (from C-4 to C-18) between compound **1** and pestalpolyol B verified the integrity of this partial skeleton (Figure 2). Thus, the whole structure of **1** was elucidated and named (4*E*,8*E*,12*E*,16*E*)-3,7,11,15-tetrahydroxy-4,6,8,10,12,14,16-heptamethyloctadeca-4,8,12,16-tetraenoic acid.

Compound **2** was also obtained as a colorless oil. Its molecular formula was established as $C_{20}H_{34}O_6$ via HRESIMS analysis. Compound **2** exhibited a highly similar structural framework to compound **1**. The partially similar HMBC and ^{1}H-^{1}H COSY correlations spanning the C-3 to C-11 moiety confirmed the conserved aliphatic chain structure in compound **2** (Figure 2). Compared with compound **1**, an additional methyl group was attached to C-2 in compound **2**, verified by the HMBC cross-peaks from H_3-15 to C-1, C-2, and C-3 and the ^{1}H-^{1}H COSY correlation between H-2 and H-3. Another distinction from compound **1** was that the double-bond moiety at the C-12–C-13 in compound **1** was replaced by an epoxide group in compound **2**, accompanied by the loss of the fragment spanning C-18–C-24. Specifically, the HMBC cross-peaks from H_3-20 to C-12 and C-13, and from H_3-14 to C-12, coupled with the ^{1}H-^{1}H COSY correlation between H_3-14 and H-13, definitively corroborated the existence of the 2,3-dimethyloxirane ring system (Figure 2), and the characteristic chemical-shift values of C-12 (δ_C 62.2) and C-13 (δ_H 2.84, δ_C 56.6) supported the formation of an epoxide moiety. The HMBC cross-peaks from H-20 to C-11 and C-12 established the C-11–C-12 linkage, which ultimately validated the overall structure of compound **2**, which was named (4*E*,8*E*)-11-(2,3-dimethyloxiran-2-yl)-3,7,11-trihydroxy-2,4,6,8,10-pentamethylundeca-4,8-dienoic acid.

Unfortunately, the relative and absolute configurations of compounds **1** and **2** (Figure 1) could not be unambiguously assigned merely based on the available NMR spectroscopic data. Multiple attempts to obtain single crystals suitable for X-ray diffraction analysis were unsuccessful.

Table 2. Antimicroalgal activity of asperpolyols A and B (**1** and **2**) (IC_{50}, μg/mL)

Compounds	*H. akashiwo*	*P. donghaiense*	*P. micans*
1	86.9 ± 7.8	84.2 ± 4.7	62.8 ± 1.7
2	_[a]	_[a]	_[a]
$CuSO_4$	5.9 ± 0.1	2.5 ± 0.2	2.1 ± 0.2

Note: [a] IC_{50} > 128 μg/mL.

Asperpolyols A and B (**1** and **2**) were evaluated for their antimicroalgal activities against three marine phytoplankton species, namely *Heterosigma akashiwo*, *Prorocentrum donghaiense*, and *Prorocentrum micans*. As presented in Table 2, compound **1** displayed weak activity against *H. akashiwo*, *P. donghaiense*, and *P. micans* with IC_{50} values of 86.9 μg/mL, 84.2 μg/mL, and 62.8 μg/mL, respectively. In contrast, compound **2** showed no significant inhibition at the highest tested concentration of 128 μg/mL on any of the tested marine phytoplankton species.

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Author Contributions

Pei Xie: Writing-original draft, Methodology, Investigation. Xiaorui Liang: Writing-review & editing, Supervision, and Data curation. Xiao Li: Methodology and Investigation. Zhenzhen Shi: Writing-original draft.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Artificial Intelligence (AI) Statement

The authors affirm that no Artificial Intelligence (AI) tools were employed in the preparation of this manuscript, which includes language editing, graphical abstract preparation, figure generation, or image editing.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

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