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Xylarinic Acids A and B, New Antifungal Polypropionates from the Fruiting Body of *Xylaria polymorpha*

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Abstract Two new polypropionates designated as xylarinic acids A and B were isolated from the fruiting body of *Xylaria polymorpha*. Their structures were established as 4,6,8-trimethyl-2,4-decadienoic acid and 2,4,6-trimethyl-2-octenoic acid, respectively, on the basis of extensive spectroscopic analysis. Both compounds displayed significant antifungal activity against plant pathogenic fungi *Pythium ultinum*, *Magnaporthe grisea*, *Aspergillus niger*, *Alternaria panax*, and *Fusarium oxysporium*, whereas they did not show antibacterial and cytotoxic effect.

Keywords *Xylaria polymorpha*, mushroom, xylarinic acid, polypropionate, antifungal activity

Introduction

Mushrooms produce a large variety of secondary metabolites with unique chemical structures and interesting biological activities. In the course of screening for antimicrobial agents from our mushroom extract library (about 300 species of mushrooms), we found that the extract of the fruiting body of *Xylaria polymorpha* exhibited potent antifungal activity against the plant pathogenic fungi. *Xylaria*, belonging to the Ascomycotina, is known to produce diverse classes of bioactive compounds including cytochalasin analogs [1], antifungal metabolites multiplolides A and B [2], NPY Y5 receptor antagonists xyarenals A and B [3], acetylcholine esterase inhibitors xyloketals A~E [4], xylariamide A [5], and xanthones [6].

In this study, two new polypropionates, xylarinic acids A (**1**) and B (**2**) (Fig. 1), have been isolated from the extract of the mushroom *X. polymorpha* by using antifungal activity-guided fractionation. We herein describe the isolation, structure determination, and antifungal activity of these compounds.

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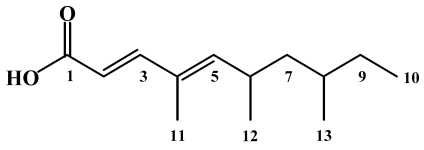
Materials and Methods

General Experimental Procedures

Optical rotation was determined using a JASCO P-1020 polarimeter. EI-MS and high resolution EI-MS were taken on a JMS-700 JEOL mass spectrometer. UV and IR spectra were recorded on a Shimadzu UV-300 and FT-IR Equinox 55 spectrometer, respectively. NMR spectra were obtained



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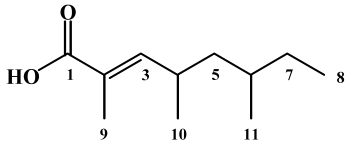

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Fig. 1 Structures of xylarinic acids A (**1**) and B (**2**).

Fig. 2 Photograph of the fruiting bodies of *Xylaria polymorpha*.

on a Varian UNITY Inova NMR spectrometer with ^{1}H-NMR at 400 MHz and ^{13}C-NMR at 100 MHz in $CDCl_3$. Chemical shifts are given in ppm (δ) using TMS as internal standard.

Mushroom Material

The dried mushroom *X. polymorpha* (285 g) was collected in the Gwangneung forest in Gyeonggi province, Korea, in October 2006, and identified by the staff at the Korea Research Institute of Bioscience and Biotechnology (KRIBB), according to the taxonomic key of Imazeki and Hongo [7].

Antimicrobial Activity

Antimicrobial activity was determined by the conventional paper disk (Advantec, 8 mm in diameter) method. The test microorganisms including 12 phytopathogenic fungi (*Pythium ultinum*, *Fusarium oxysporium*, *Magnaporthe grisea*, *Aspergillus niger*, *Alternaria panax*, *Phytophthora capsici*, *Alternaria mali*, *Alternaria porri*, *Botrytis cinerea*, *Rhizoctonia solani, Fulvia fulva*, *Cylindrocarpon destructans*) and three bacteria (*Salmonella sendai*, *Staphylococcus aureus*, *Bacillus subtilis*) were supplied from the Korean Collection for Type Cultures (KCTC) in the KRIBB, Korea. The paper disks containing each of 50 μg sample were placed on agar plate inoculated with the test organisms. Antibiotic activity was assessed by measuring the diameter of inhibition zone after incubation of 24 hours at 37°C for bacteria, whereas after incubation of 2~7 days at 27°C for fungi.

Cytotoxicity

The cytotoxicity was determined by measuring the reduction product of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) [8]. In brief, cells were pre-cultured in 96-well plates with 180 μl of DMEM containing 5.0% FBS for 24 hours. This was then added to diluted sample solution to a final volume of 200 μl and cultured under 5.0% CO_2 at 37°C for 48 hours. MTT was dissolved in phosphate-buffered saline and added to the cell culture. After 2 hours, the medium was removed and the remaining MTT crystals were dissolved in 100 μl of DMSO. The absorbance was measured at 570 nm with a background correction at 690 nm.

Results

Isolation and Purification of Xylarinic Acids A and B

The ground fruiting bodies of *X. polymorpha* were extracted twice with MeOH at room temperature for 2 days. After removal of MeOH under reduced pressure, the concentrate was partitioned between hexane and H_2O and then $CHCl_3$ and H_2O. The concentrated hexane-soluble portion was subjected to a column of silica gel and eluted with increasing amount (2.0%, 5.0%, 10%, 20%, and 50%, stepwise) of EtOAc in hexane to give two antifungal fractions. One was chromatographed on a column of Sephadex LH-20 with $CHCl_3$: MeOH (1 : 1, v/v) to give **1** (200 mg). The other was purified by ODS column chromatography with 70% aqueous MeOH to afford **2** (1.5 mg).

Physico-chemical Properties

1 was obtained as a colorless oil and was soluble in $CHCl_3$, DMSO, and MeOH and insoluble in H_2O. Its molecular formula was determined to be $C_{13}H_{22}O_2$ by high-resolution EI-MS measurement (found 210.1620, calcd 210.1620). The UV spectrum of **1** showed absorption maxima at 262 nm, which can be ascribed to an $\alpha,\beta,\gamma,\delta$-unsaturated carbonyl group, and 202 nm. The IR spectrum of **1** exhibited absorption bands due to hydroxyl (3440 cm^{-1}) and α,β-unsaturated carbonyl (1690 cm^{-1}) groups. **1** has an optical rotation value of α_D^{25} −69.6 (*c* 10, MeOH). **2** was also obtained as a colorless oil of small amount, and its molecular formula, $C_{11}H_{20}O_2$, was determined by high-resolution EI-MS measurement (found 184.1459, calcd 184.1463). The UV spectrum of **2** exhibited an absorption maximum at 214 nm, which can be ascribed to an α,β-unsaturated carbonyl group. **2** has an optical rotation value of α_D^{25} −25.0 (*c* 0.1, MeOH).

Structure Determination

Chemical structures of **1** and **2** were determined by NMR spectroscopic analysis. The ^{1}H-NMR spectrum showed the signals due to three olefinic methine protons at δ 7.38,

Table 1 ^{1}H- and ^{13}C-NMR spectral data of compounds xylarinic acids A (**1**) and B (**2**) in $CDCl_3$[a]

No.	1		2	
	δ_C	δ_H	δ_C	δ_H
1	173.6		172.7	
2	114.9	5.76 (1H, d, *J*=15.6)[b]	125.4	
3	152.6	7.38 (1H, d, *J*=15.6)	151.3	6.66 (1H, dd, *J*=10.0, 1.2)
4	131.4		31.3	2.64 (1H, m)
5	150.4	5.67 (1H, d, *J*=10.0)	44.2	1.38 (1H, m)
				1.16 (1H, m)
6	31.2	2.64 (1H, m)	32.5	1.27 (1H, m)
7	44.6	1.30 (1H, m)	30.2	1.30 (1H, m)
		1.09 (1H, m)		1.16 (1H, m)
8	32.6	1.23 (1H, m)	11.4	0.86 (3H, t, *J*=7.2)
9	29.9	1.30 (1H, m)	12.3	1.87 (3H, d, *J*=1.2)
		1.13 (1H, m)		
10	11.5	0.82 (3H, t, *J*=7.2)	20.6	1.01 (3H, d, *J*=6.4)
11	12.5	1.79 (3H, s)	19.2	0.84 (3H, d, *J*=6.0)
12	21.2	0.97 (3H, d, *J*=6.4)		
13	19.2	0.80 (3H, d, *J*=6.4)		
OH		11.82 (1H, br s)		

[a] NMR data were measured at 400 MHz for proton and at 100 MHz for carbon.

[b] Proton resonance integral, multiplicity, and coupling constant (*J*=Hz) are in parentheses.

5.76, and 5.67, two methylenes, two methines, and four methyl protons at δ 1.79, 0.97, 0.82, and 0.80 (Table 1). In the ^{13}C-NMR spectrum, a carbonyl carbon, three sp^2 methine carbons, one sp^2 quaternary carbon, two sp^3 methine carbons, two sp^3 methylene carbons, and four methyl carbons were evident. The ^{1}H-^{1}H COSY experiment established a diene moiety and a partial structure of C-5 to C-10, suggesting that **1** has a polypropionate moiety (Fig. 3). The structure of **1** was determined by the HMBC experiment, which showed the long-range correlations from H-2 to C-1 and C-4, from H-3 to C-1 and C-5, from H-5 to C-3, C-7, C-11, and C-12, from H-6 to C-4 and C-8, from H-7 to C-9, C-12, and C-13, from H-10 to C-8 and C-9, from H-11 to C-3, C-4, and C-5, from H-12 to C-5, C-6, and C-7, from H-13 to C-7, C-8, and C-9, as shown in Fig. 3. The *E* configuration of the double bonds of C-2 and C-4 was assigned on the basis of the proton coupling constants of 15.6 Hz and carbon chemical shift of C-11 having γ-effect, respectively. We planned to determine the stereochemistry of **1** at C-6 and C-8 by oxidative degradation with $NaIO_4$ and $KMnO_4$ and comparison of the optical rotation value of the reaction product, 2,4-dimethylhexanoic acid, with its reference data [9]. But unfortunately, we were not able to isolate the reaction product 2,4-dimethylhexanoic acid. Thus, the stereochemistry of **1** still remains to be determined.

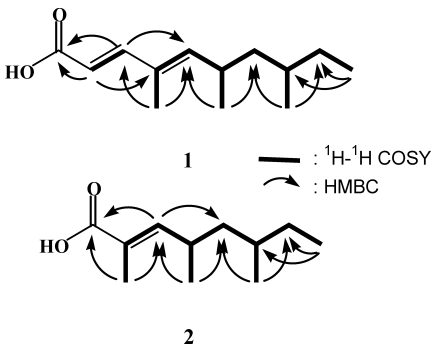

Fig. 3 ^{1}H-^{1}H COSY and HMBC correlations of compounds **1** and **2**.

The structure of **2** was established by comparing its NMR data with those of **1**. The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**, except that the signals due to the two olefinic methines were missing. In the ^{1}H-^{1}H COSY spectrum, the methine proton at δ 2.64 showed a cross peak to the methine proton at δ 6.66 that was, in turn, correlated to the vinylic methyl protons at δ 1.87 by allylic coupling. The structure of **2** was confirmed by the HMBC experiment, which showed the long-range correlations

from H-8 to C-6 and C-7, from H-9 to C-1, C-2, and C-3, from H-10 to C-3, C-4, and C-5, and from H-11 to C-5, C-6, and C-7. Accordingly, the structure of **2** was established as 2,4,6-trimethyl-2-octenoic acid with undetermined stereochemistry at C-4 and C-6.

Antimicrobial Activity and Cytotoxicity

To date, many structurally-novel polypropionate metabolites have been isolated from marine bacteria and sponges, of which Mollusca was the most important source. Most members of this class have been reported to be against Gram-positive bacteria, yeast, and human cancer cell lines [10]. Interestingly, **1** and **2** exhibited potent antifungal activity (clear zone diameters of 16∼20 mm) against *Pythium ultinum* and *Magnaporthe grisea*, moderate activity (clear zone diameters of 11∼15 mm) against *Aspergillus niger*, *Alternaria panax*, and *Fusarium oxysporium*, and marginal activity against *Phytophthora capsici*, *Alternaria mali*, *Alternaria porri*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fulvia fulva*, and *Cylindrocarpon destructans*, whereas they showed no activity against bacteria. In *in vitro* cytotoxicity of **1** and **2** estimated by MTT assay [8], these compounds exhibited no cytotoxic effect up to 200 μM against the cancer cell lines, A549 (lung adenocarcinoma), HT-1080 (fibrosarcoma), and SW 620 (colorectal adenocarcinoma).

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