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NOTE

Xylarinols A and B, two new 2-benzoxepin derivatives from the fruiting bodies of *Xylaria polymorpha*

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Basidiomycetes, as decomposers of forest litter, represent an ecologically important group of organisms in the environment, and are known to produce a large variety of secondary metabolites with unique chemical structures and interesting biological activities.[1] The genus *Xylaria* has been known to produce a diverse class of bioactive compounds, including cytochalasin analogs with chemokine receptor antagonistic activity and cytotoxicity,[2] multiplolides A, B and xylariamide A with antifungal activity,[3,4] xylarenals A and B with neuropeptide Y receptor antagonistic activity[5] and xyloketals A–E, acetylcholinesterase inhibitors.[6] Earlier, we reported two antifungal substances, xylarinic acids A and B, from the methanolic extract of *Xylaria polymorpha*.[7] Our ongoing investigation for novel chemical constituents from *X. polymorpha* has resulted in the isolation of two new 2-benzoxepin derivatives with ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate)) radical scavenging activity. Benzoxepin is very rare in naturally occurring compounds. In this paper, we describe the isolation and structure determination of xylarinols A (**1**) and B (**2**), and their biological activity.

Xylarinols were isolated from the fruiting bodies of *X. polymorpha*, as shown in Figure 1. The collected fruiting bodies were ground and then extracted twice with methanol (MeOH) at room temperature for 2 days. After removal of MeOH under reduced pressure, the concentrate was partitioned between chloroform and water and then ethyl acetate and water. The ethyl acetate-soluble portion was chromatographed on a column of silica gel and eluted with increasing amounts (2.0, 5.0, 10, 20 and 50%, stepwise) of MeOH in $CHCl_3$ to give two fractions, which exhibited moderate ABTS radical scavenging activity. One was purified by Sephadex LH-20 column chromatography with $CHCl_3$–MeOH (1 : 1, v/v), followed by preparative reversed-phase HPLC with 40% aqueous MeOH at a flow rate of 6.0 ml min^{-1} to yield xylarinol A (**1**, 1.0 mg). The other fraction was purified by preparative reversed-phase HPLC with 30% aqueous MeOH at a flow rate of 6.0 ml min^{-1} to provide xylarinol B (**2**, 1.7 mg).

Xylarinol A was isolated as a white powder and showed a molecular ion peak at *m/z* 176 in the electron impact mass measurement. Its high-resolution electron impact mass measurement provided an accurate mass at *m/z* 176.0472 [M^+, Δ−0.1 mmu], establishing its molecular formula as $C_{10}H_8O_3$. The UV spectrum in MeOH exhibited absorption maxima at 205 (log ε 4.75), 217 (log ε 4.63), 277 (log ε 4.43) and 316 (log ε 4.07) nm. The IR spectrum suggested the presence of a hydroxyl group (3444 cm^{-1}) and an α,β-unsaturated ester group (1651 cm^{-1}). The ^{1}H-NMR spectrum showed signals due to 1,2,3-trisubstituted benzene ring at δ 7.28, 6.95 and 6.94, two olefinic methine peaks assigned to a *cis*-1,2-disubstituted double bond unit at δ 7.31 (*J*=12.0 Hz) and 6.29 (*J*=12.0 Hz), and a methylene peak at δ 5.24. In the ^{13}C-NMR spectrum, an ester carbonyl carbon at δ 169.6, an oxygen-bearing sp^2 carbon at δ 154.9, five sp^2 methines, two sp^2 quaternary carbons and an oxymethylene at δ 61.4 were evident (Table 1). The ^{1}H–^{1}H COSY spectrum revealed two partial structures, and the heteronuclear multiple quantum correlation spectrum established the proton-bearing carbons, as shown in Figure 2. The structure of **1** was unambiguously determined by the heteronuclear multiple bond correlation spectrum. The long-range correlations from H-6 to C-8 and C-9a, and from H-8 to C-6 and C-9a revealed the presence of 2,3-disubstituted-phenol moiety in **1**. The oxepinone ring system was determined by the heteronuclear multiple bond correlations of H-1 to C-3 (δ 169.6), C-5a (δ 137.3) and C-9a (δ 121.7), of H-4 to C-3 (δ 169.6) and C-5a (δ 137.3), and of H-5 to C-9a (δ 121.7). Finally, the heteronuclear multiple bond correlations from H-1 to C-9 (δ 154.9) and from H-5 to C-6 (δ 120.7) completed the structure of **1** as shown in Figure 2. Therefore, the structure of **1** was determined to be 9-hydroxy-1*H*-benzo[*c*]oxepin-3-one, a new benzoxepin derivative.

Xylarinol B was obtained as a yellow powder with a specific rotation value of −3.32 (*c* 0.1, MeOH). The molecular formula of **2** was established to be $C_{12}H_{16}O_4$ by the high-resolution EI-MS providing a molecular ion peak at *m/z* 224.1050 [M^+, Δ+0.1 mmu]. The UV

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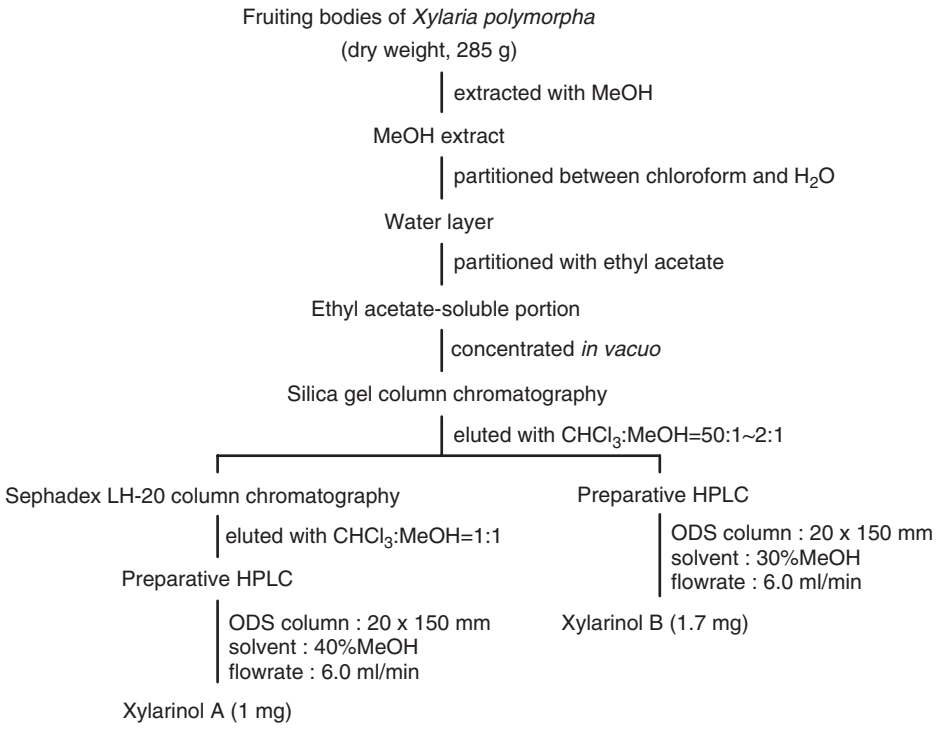

Figure 1 Purification procedures of xylarinol A (**1**) and B (**2**).

Table 1 ^{1}H- and ^{13}C-NMR spectral data of xylarinols A (1) and B (2)

Positions	*Xylarinol A*		*Xylarinol B*	
	δ_H	δ_C	δ_H	δ_C
1a	5.24 (2H, s)[a]	61.4	5.05 (1H, dd, *J*=12.0, 2.8)	71.3
1b			4.95 (1H, d, *J*=12.0)	
3		169.6	3.70 (1H, m)	73.5
4	6.29 (1H, d, *J*=12.0)	121.6	1.82 (2H, m)	40.5
5	7.31 (1H, d, *J*=12.0)	141.7	5.41 (1H, m)	82.6
5a		137.3		145.7
6	6.95 (1H, d, *J*=7.8)	120.7	6.67 (1H, d, *J*=7.8)	113.0
7	7.28 (1H, t, *J*=7.8)	130.1	7.10 (1H, t, *J*=7.8)	130.2
8	6.94 (1H, d, *J*=7.8)	117.1	6.64 (1H, d, *J*=7.8)	114.8
9		154.9		152.9
9a		121.7		126.1
1′			3.61 (1H, m)	72.0
2′			1.16 (3H, d, *J*=6.4)	18.6

NMR spectra were measured in CD_3OD (25 °C) at 400 MHz for ^{1}H and 100 MHz for ^{13}C.
[a]Integral, multiplicity and coupling constants in Hz are given in parentheses.

spectrum in MeOH exhibited absorption maxima at 211 (log ε 3.93), 222 (log ε 3.87), 269 (log ε 3.29) and 276 (log ε 3.28) nm, and its IR spectrum suggested the presence of a hydroxyl group at 3433 cm^{-1}. The ^{1}H-NMR spectrum exhibited signals assignable to 1,2,3-trisubstituted benzene at δ 7.10, 6.67 and 6.64, three oxygenated methines at δ 5.41, 3.70 and 3.61, one nonequivalent oxygenated methylene at δ 5.05 and 4.95, one methylene at δ 1.82, and one methyl at δ 1.16. The ^{13}C-NMR spectrum showed 12 carbons, which were identified as an oxygen-bearing sp^2 carbon at δ 152.0, three sp^2 methines, two sp^2 quaternary carbons, three oxymethines, one oxymethylene, one methylene and one methyl by the DEPT spectrum. Two partial structures were established on the basis of the proton multiplicity and *J* values, as well as ^{1}H–^{1}H COSY spectral data, as shown in Figure 2. The proton at δ 5.05 (H-1a) of the nonequivalent oxygenated methylene protons was split as a doublet of doublet by the geminal coupling of 12.0 Hz and homoallylic coupling of 2.8 Hz to H-5, which was confirmed by the COSY data. These partial structures were unambiguously connected by the heteronuclear multiple bond correlation spectrum, which showed the long-range correlations of H-1 to C-5a and C-9a, of H-4 to C-3 and C-5a, of H-6 to C-5, C-8, and C-9a, and of H-8 to C-9a, establishing the presence of benzoxepin moiety. Therefore, the structure of **2** was determined as 1,3,4,5-tetrahydro-3-(1-hydroxyethyl)benzo[*c*]oxepin-5,9-diol, a new benzoxepin derivative. The relative configuration of oxepin ring was proposed by the NOE experiments. NOE enhancements of H-1a, H-3 and H-4 by irradiation of H-5 were observed, and H-1a showed NOE with H-5, suggesting that methine protons of H-1a, H-3 and H-5 were coplanar (Figure 2).

Benzoxepin is known to be very rare in naturally occurring compounds. To date, several 1-benzoxepin and 1-benzoxepinone derivatives have been isolated from *Marsamiellus ramealis*,[8] *Mycena galopus*[9] and *Pterula* species[10,11] as antibiotics or inhibitors of NADH. We evaluated the antimicrobial activity of compounds **1** and **2** by the conventional paper disk (Advantec, Tokyo, Japan; 8 mm in diameter) method[7] at a concentration of 50 μg per disk. Fifteen 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 used. However, compounds **1** and **2** showed no antimicrobial activity against all test organisms. We also measured the ABTS radical scavenging activity of both compounds by using ABTS radical cation decolorization assay with minor modifications.[12] As a result, these compounds were found to exhibit moderate ABTS radical scavenging

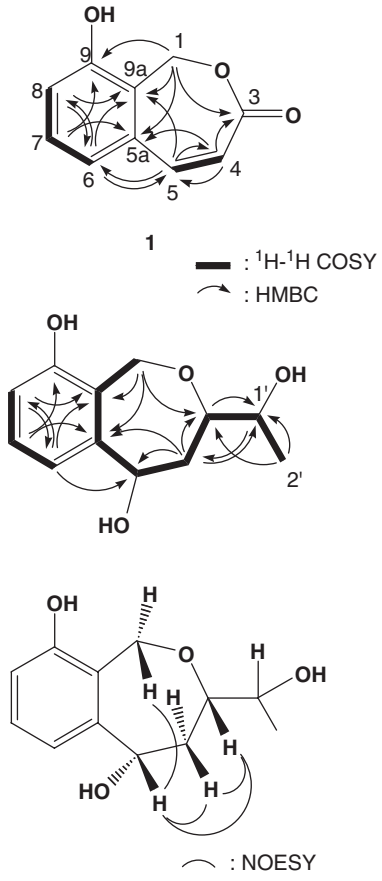

Figure 2 Structures of xylarinol A (**1**) and B (**2**) elucidated by 2-D NMR experiments.

activity with 40 and 45% inhibition, respectively, at 100 μM concentration. Compounds **1** and **2** were obtained in too small amounts for biological activity test, and so some synthetic effort is needed for further study.

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