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Metabolites from the endophytic fungus *Xylaria* sp. PSU-D14

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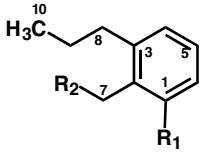
ABSTRACT

Glucoside derivatives, xylarosides A (**1**) and B (**2**), were isolated from the broth extract of the endophytic fungus *Xylaria* sp. PSU-D14 along with two known compounds, sordaricin (**3**) and 2,3-dihydro-5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one (**4**). The structures were assigned by spectroscopic methods. Sordaricin (**3**), one of the known metabolites, exhibited moderate antifungal activity against *Candida albicans* ATCC90028 with a MIC value of 32 μg/ml.

1. Introduction

Many biologically active secondary metabolites have been isolated from the genus *Xylaria*, for example, antifungal multiplolides (Boonphong et al., 2001), cytotoxic cytochalasins (Dagne et al., 1994), and acetyl choline esterase inhibitor xyloketals (Lin et al., 2001). The ethyl acetate extract from the culture broth of *Xylaria* sp. PSU-D14 exhibited antifungal activity against *Candida albicans* ATCC90028 (CA28) with a MIC value of 128 μg/ml (Phongpaichit et al., 2006). We describe herein the isolation and structural elucidation of two new glucoside derivatives (**1** and **2**) together with two known compounds, sordaricin (**3**) (Weber et al., 2005; Mander and Thomson, 2005; Hauser and Sigg, 1971) and 2,3-dihydro-5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one (**4**) (Gray et al., 1999; Dai et al., 2006). Their structures were assigned by spectroscopic methods and comparison of the ^{1}H and ^{13}C NMR spectroscopic data with those reported in the literature. All compounds were tested for antifungal activity against CA28.



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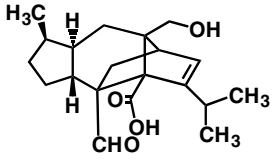
2: $R_1 = glu$, $R_2 = OH$



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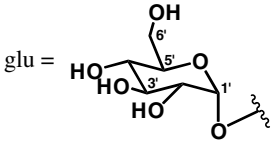

2. Results and discussion

Xylaroside A (**1**) was obtained as a colorless solid and melted at 103.1–103.6 °C with $[\alpha]_D^{27} + 27$ (*c* 0.36, MeOH). The UV spectrum showed maximum absorption bands of a benzene chromophore at λ_{max} 220 and 281 nm. The IR spectrum exhibited an absorption band at 3348 cm^{-1} for a hydroxyl group. The HR–EIMS showed the molecular formula $C_{16}H_{24}O_7$. The ^{1}H NMR spectroscopic data (Table 1) displayed three aromatic protons of a 1,2,3-trisubstituted

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Table 1
^{1}H and ^{13}C NMR spectroscopic data of xylarosides A (**1**) and B (**2**)

Position	**1**		**2**	
	(J in Hz)	δ_C (mult.)	δ_H (J in Hz)	δ_C (mult.)
1	–	157.9, qC	–	156.9, qC
2	–	122.4, qC	–	128.6, qC
3	–	144.0, qC	–	140.9, qC
4	6.70, *dd* (7.5, 1.5)	121.7, CH	6.87, *dd* (8.5, 2.0)	122.7, CH
5	7.07, *t* (7.5)	129.7, CH	7.13, *t* (8.5)	127.3, CH
6	6.71, *dd* (7.5, 1.5)	114.8, CH	7.17, *dd* (8.5, 2.0)	112.6, CH
7	a: 4.99, *d* (11.0) b: 4.61, *d* (11.0)	63.4, CH_2	a: 4.79, *d* (12.0) b: 4.56, *d* (12.0)	54.7, CH_2
8	a: 2.68, *dd* (14.0, 7.5) b: 2.62, *dd* (14.0, 7.5)	35.8, CH_2	2.70, *t* (7.5)	34.0, CH_2
9	1.58, *sextet* (7.5)	25.7, CH_2	1.58, *m*	24.0, CH_2
10	0.96, *t* (7.5)	14.4, CH_3	0.95, *t* (7.5)	12.4, CH_3
1′	4.93, *d* (4.0)	99.6, CH	5.35, *d* (4.0)	99.0, CH
2′	3.42, *dd* (9.0, 4.0)	73.4, CH	3.59, *dd* (9.5, 4.0)	71.7, CH
3′	3.61, *t* (9.0)	75.3, CH	3.91, *t* (9.5)	73.3, CH
4′	3.36, *t* (9.0)	71.9, CH	3.48, *t* (9.5)	69.7, CH
5′	3.70, *m*	73.7, CH	3.80, *m*	72.7, CH
6′	a: 3.82, *dd* (12.0, 2.5) b: 3.69, *dd* (12.0, 6.0)	62.9, CH_2	a: 3.84, *dd* (11.5, 2.0) b: 3.73, *dd* (11.5, 5.5)	60.9, CH_2

benzene [δ_H 7.07 (1H, *t*, J = 7.5 Hz), 6.71 (1H, *dd*, J = 7.5, 1.5 Hz) and 6.70 (1H, *dd*, J = 7.5, 1.5 Hz)], two nonequivalent oxymethylene protons [δ_H 4.99 (1H, *d*, J = 11.0 Hz) and 4.61 (1H, *d*, J = 11.0 Hz)], one propyl side chain [δ_H 2.68 (1H, *dd*, J = 14.0, 7.5 Hz), 2.62 (1H, *dd*, J = 14.0, 7.5 Hz), 1.58 (2H, *sextet*, J = 7.5 Hz) and 0.96 (3H, *t*, J = 7.5 Hz)] and characteristic signals of a glucose moiety: one anomeric proton (δ_H 4.93, *d*, J = 4.0 Hz), two nonequivalent oxymethylene protons [δ_H 3.82 (1H, *dd*, J = 12.0, 2.5 Hz) and 3.69 (1 H, *dd*, J = 12.0, 6.0 Hz)] and four oxymethine protons [δ_H 3.70 (1H, *m*), 3.61 (1H, *t*, J = 9.0 Hz), 3.42 (1H, *dd*, J = 9.0, 4.0 Hz) and 3.36 (1H, *t*, J = 9.0 Hz)]. The appearance of the anomeric proton as a *doublet* with a small coupling constant of 4.0 Hz indicated that the glucose unit must be an α-glucopyranose. The aromatic protons at δ_H 6.70, 7.07 and 6.71 were assigned as H-4, H-5 and H-6, respectively, on the basis of their multiplicity and their coupling constants. ^3J HMBC correlations (Table 2) from H_{ab}-8 (δ_H 2.68 and 2.62) of the propyl side chain to C-2 (δ_C 122.4) and C-4 (δ_C 121.7) and signal enhancement of H_{ab}-8 after irradiation of H-4 in the NOEDIFF experiment established the attachment of the propyl group at C-3 (δ_C 144.0). The nonequivalent oxymethylene protons, H_{ab}-7 (δ_H 4.99 and 4.61), gave HMBC crosspeaks with C-1′ (δ_C 99.6) of the α-glucopyranose moiety, C-1 (δ_C 157.9), C-2 and C-3, thus connecting this group with C-2 of the 1,2,3-trisubstituted benzene and forming an ether linkage with C-1′ of the glucose unit. Signal enhancement of both H-1′ and H_{ab}-8 upon irradiation of H_{ab}-7 in the NOEDIFF experiment supported the assigned location. The substituent at C-1 must be a hydroxyl group according to its ^{13}C chemical shift. Thus, compound **1** was determined as a new naturally occurring α-glucoside.

Table 2
HMBC correlations of xylarosides A (**1**) and B (**2**)

Position	HMBC correlations	
	1	**2**
4	C-3, C-5, C-6, C-8	C-2, C-3, C-5, C-6, C-8
5	C-1, C-3, C-4	C-1, C-3, C-4
6	C-1, C-2, C-4, C-5	C-1, C-2, C-4
7	C-1, C-1′, C-2, C-3	C-1, C-2, C-3
8	C-2, C-3, C-4, C-9, C-10	C-2, C-3, C-4, C-9, C-10
9	C-3, C-8, C-10	C-3, C-8, C-10
10	C-8, C-9	C-8, C-9
1′	C-2′, C-5′, C-7	C-1, C-2′, C-5′
2′	C-3′	C-3′, C-4′
3′	C-2′, C-4′	C-2′, C-4′, C-5′
4′	C-5′, C-6′	C-3′, C-5′, C-6′
5′	C-3′	C-4′, C-6′
6′	C-4′, C-5′	C-5′

Xylaroside B (**2**) was obtained as a colorless solid and melted at 103.4–104.1 °C with $[\alpha]_D^{27}$ + 35 (*c* 0.20, MeOH). The UV and IR spectra were almost identical to those of **1**. The HR–EIMS showed that they had the same molecular formula. The significant difference in the ^{1}H NMR spectrum was the chemical shift of H-1′ of the α-glucose moiety (δ_H 4.93 in **1** and δ_H 5.35 in **2**). The ^3J HMBC cross peak (Table 2) from H-1′ to C-1 (δ 156.9) established an ether linkage between the anomeric carbon (δ_C 99.0, C-1′) and the oxyaromatic carbon (C-1), but not the oxyaliphatic C-7 as found in **1**. The irradiation of H-1′ of the α-glucopyranose unit enhanced signal intensity of H-6 (δ_H 7.17, 1H, *dd*, J = 8.5, 2.0 Hz), thus supporting this assignment. The hydroxymethyl protons [H_{ab}-7, δ_H 4.79 (1H, *d*, J = 12.0 Hz) and 4.56 (1H, *d*, J = 12.0 Hz)] and the propyl side chain were located at C-2 (δ_C 128.6) and C-3 (δ_C 140.9), respectively, on the basis of ^3J HMBC correlations of H_{ab}-7/C-1 and C-3 and those of H_2-8 (δ_H 2.70, *t*, J = 7.5 Hz)/C-2 and C-4 (δ_C 122.7). Therefore, compound **2** differed from **1** in the location of the glucose unit.

Among the isolated metabolites, sordaricin (**3**), the diterpene aglycone of potent antifungal sordarins, gave the best antifungal activity against CA28 with the MIC value of 32 μg/ml. However, it was much less active than amphotericin B (MIC value of 0.25 μg/ml). The remaining compounds showed very weak antifungal activity with equal MIC value (>128 μg/ml).

3. Concluding remarks

Two new glucoside derivatives (**1** and **2**) were isolated from the fungus *Xylaria* sp. PSU-D14 together with two known compounds **3** and **4**. Additionally, sordaricin and sordarins exhibit antifungal activity against a wide range of fungal pathogens (Hauser and Sigg, 1971; Cuevas et al., 1999; Quesnelle et al., 2003), and sordaricin derivatives have been isolated from a range of ascomycetes but only *Podospora pleiospora* produced natural sordaricin (Weber et al., 2005). In this study, it was demonstrated that *Xylaria* sp. produces not only a sordaricin derivative, xylarin (Schneider et al., 1995), but also sordaricin.

4. Experimental

4.1. General experimental procedures

Melting points were measured on an electrothermal melting point apparatus (Electrothermal 9100). Infrared spectra (IR) were recorded as neat on a Perkin Elmer 783 FTS165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in methanol on a SHIMADZU UV-160A spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz Bruker FTNMR Ultra Shield™ spectrometer, with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained on a MAT 95 XL mass spectrometer (Thermofinnigan). Optical rotations were measured in methanol solution on a JASCO P-1020 polarimeter. Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was carried out on silica gel (Merck) type 100 (70–230 Mesh ASTM) with a gradient system of CH_2Cl_2/MeOH or on Sephadex LH20 with MeOH, unless otherwise stated.

4.2. Fungal material

The endophytic fungus *Xylaria* sp. PSU-D14 was isolated from the leaves of *Garcinia dulcis*, collected in Songkhla Province,

Thailand, in 2005. This fungus was deposited as PSU-D14 (GenBank Accession Number DQ480352) at the Department of Microbiology, Faculty of Science, Prince of Songkla University.

4.3. Fermentation and Isolation

The endophytic fungus *Xylaria* sp. PSU-D14, grown on potato dextrose agar (PDA) at 25 °C for 5 d, was inoculated into 500 ml Erlenmeyer flasks containing 300 ml potato dextrose broth (PDB) at room temperature for 4 weeks. The cultures were separated by filtration into the mycelia and filtrate. The filtrate was extracted three times with an equal volume of EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure to obtain a brown gum (650 mg). The crude extract was fractionated by CC over Sephadex LH20 to afford five fractions (A–E). Fraction B (204 mg) was subjected to CC over Sephadex LH20 to give three fractions (B1–B3). Fraction B2 was separated by CC over silica gel to give five subfractions (B21–B25). Subfraction B22 contained **3** (32.3 mg). Subfraction B24 (24.5 mg) was further purified by CC over silica gel to give four subfractions. The second subfraction contained **1** (4.8 mg) while the third subfraction (12.1 mg) was subjected to CC over Sephadex LH20 to give **2** (1.5 mg). Fraction D gave **4** (46.2 mg).

4.3.1. Xylaroside A (*1*)

Colorless solid; 103.1–103.6 °C; $[\alpha]_D^{27} + 27$ (*c* 0.36, MeOH); UV λ_{max}^{MeOH} nm (logε): 220 (3.45), 281 (2.95); FT-IR ν_{max}^{neat} cm^{-1} 3348, 1609, 1588; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; EIMS *m*/*z* (% relative intensity): 328 (1), 297 (12), 149 (100), 133 (21), 121 (34); HR–EIMS *m*/*z* 328.1530 $[M]^+$ (calcd for $C_{16}H_{24}O_7$: 328.1522).

4.3.2. Xylaroside B (*2*)

Colorless solid; 103.4–104.1 °C; $[\alpha]_D^{27} + 35$ (*c* 0.20, MeOH); UVλ_{max}^{MeOH} nm (logε): 217 (4.53), 276 (3.87); FT-IR ν_{max}^{neat} cm^{-1} 3344, 1602, 1584; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; EIMS *m*/*z* (% relative intensity) 328 (1), 166 (35), 148 (100), 133 (11); HR–EIMS *m*/*z* 328.1529 $[M]^+$ (calcd for $C_{16}H_{24}O_7$: 328.1522).

4.4. Antifungal assay

The minimum inhibitory concentrations (MICs) were determined by agar dilution assay (Lorian, 1996). The test microorganism was CA28. The crude extract was dissolved in DMSO. Serial 2-fold dilutions of the test extract were mixed with melted sabouraud's dextrose agar (SDA) medium in the ratio of 1:50 in microtiter plates with flat-bottomed wells. Final concentrations in agar ranged between 128 and 0.25 μg/ml for the crude extract and pure compounds. Inoculum suspensions were spotted on the extract/compound amended agar surface (10^4 CFU per spot). The inoculated plates were incubated at 35 °C for 24 h. MICs were recorded by reading the lowest concentrations that inhibited visible growth. Growth controls were performed on agar containing DMSO. Amphotericin B was used as positive control.

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