An [11]Cytochalasin Derivative from the Marine-Derived Fungus *Xylaria* sp. PSU-F100

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A new [11]cytochalasin derivative, xylarisin (1), was isolated from the marine-derived fungus *Xylaria* sp. PSU-F100 along with six known metabolites: three mellein derivatives (2—4), one pyrone derivative (5) and two carboxylic acids (6,7). The structure and stereochemistry of 1 were determined by NMR and X-ray diffraction analyses. All isolated compounds showed mild antibacterial activity against standard *Staphylococcus aureus* ATCC 25923 and methicillin-resistant strain.

Key words *Xylaria* sp.; marine-derived fungus; cytochalasin; antibacterial activity

Research concerning the investigation of metabolites from fungi in the genus *Xylaria* has shown that these fungi produced a wide range of secondary metabolites. Some of them exhibited interesting biological activities such as anti-herpes simplex virus-type 1,[1)] antifungal[2)] and anti-human immunodeficiency virus (HIV)-1 integrase[3)] activities. We found that the ethyl acetate extract from the broth of *Xylaria* sp. PSU-F100 exhibited antibacterial activity against the standard *Staphylococcus aureus* ATCC 25923 (SA). We report herein the isolation and structure determination of one new [11]cytochalasin derivative, xylarisin (**1**), together with six known metabolites: three mellein derivatives: (*R*)-(−)-mellein methyl ether (**2**),[4)] (*R*)-(−)-5-carboxymellein (**3**)[5)] and (*R*)-(−)-5-hydroxymethylmellein (**4**),[5)] one pyrone derivative: 6-[(1*R*)-1-hydroxypentyl]-4-methoxy-2*H*-pyran-2-one (**5**)[6)] and two carboxylic acids: (2*E*,4*S*)-2,4-dimethyloct-2-enoic acid (**6**)[7)] and piliformic acid (**7**).[8,9)] Compound **6** was isolated from a natural source for the first time. Their antibacterial activity against SA and methicillin-resistant *S. aureus* (MRSA) was examined.

The marine-derived fungus *Xylaria* sp. PSU-F100 was isolated from the gorgonian sea fan (*Annella* sp.). It was identified based on the analyses of the ribosomal transcribed spacer (ITS) regions. Its ITS sequence (EU714398) is well placed in the *Xylaria* subclade comprising *Xylaria* sp. (DQ322167, DQ322165, DQ322168, EU214582) and *Xylocoremium* sp. (AM749930, AM749929) with high statistical support (100%) and sequence similarity between 95.7 to 98.3%. It was then identified as *Xylaria* sp.

Compounds **2**—**7** were identified by comparison of the ^{1}H- and ^{13}C-NMR data with those previously reported in the literature. The absolute configurations of compounds **2**—**6** were identical to those of the known ones on the basis of their similar specific rotations. For compound **7**, a chiral center was proposed to have an *R* configuration by comparing its specific rotation, $[\alpha]_D^{25}$ −118.3 (c=0.5, $CHCl_3$), with that of compound **8**, $[\alpha]_D$ −114 ($CHCl_3$).[10)]

Xylarisin (**1**) was obtained as colorless crystals. The molecular formula was determined to be $C_{22}H_{33}NO_5$ by HR-EI-MS, indicating seven double bond equivalents. The IR spectrum exhibited characteristic absorption bands for a hydroxyl (3377 cm^{-1}), an amino (3216 cm^{-1}), a ketone carbonyl (1703 cm^{-1}) and an amide carbonyl (1693 cm^{-1}). The ^{13}C-NMR spectrum (Table 1) contained 22 carbons and confirmed the presence of one ketone carbonyl (δ 210.4) and one amide carbonyl (δ 174.2). These data implied that **1** consisted of four ring systems, apart from two carbonyls and one double bond. The ^{1}H-NMR spectrum (Table 1) consisted of signals for two *trans*-olefinic protons (δ 6.51, 5.31), one amide proton (δ 6.30), one isobutyl unit (δ 1.57, 1.24, 0.94,

Table 1. NMR Spectral Data of Xylarisin (**1**) in $CDCl_3$

Position	^{1}H δ (mult., *J*, Hz)	^{13}C δ (mult.)	HMBC
1	—	64.7 (C)	
2	—	210.4 (C)	
3	a: 4.03 (dd, 18.3, 7.8)	44.6 (CH_2)	C-2, C-4, C-5
	b: 2.07 (dd, 18.3, 4.8)		C-2, C-4, C-5
4	4.18 (m)	66.7 (CH)	
5	a: 1.70 (m)	34.2 (CH_2)	C-4
	b: 1.37 (m)		C-4
6	1.68 (m)	16.2 (CH_2)	
7	a: 2.03 (m)	34.0 (CH_2)	
	b: 1.24 (m)		
8	4.08 (td, 10.5, 4.5)	75.6 (CH)	C-10
9	5.31 (dd, 15.3, 10.5)	136.8 (CH)	C-1, C-7, C-8, C-11, C-12
10	6.51 (dd, 15.3, 10.5)	129.2 (CH)	C-1, C-8, C-11, C-12
11	2.16 (dd, 10.5, 6.0)	49.6 (CH)	C-2, C-9, C-10, C-12, C-15, C-18
12	3.10 (dd, 6.0, 4.5)	54.1 (CH)	C-10, C-11, C-13
13	2.86 (dd, 6.0, 4.5)	54.6 (CH)	C-12, C-14, C-23
14	1.75 (m)	33.9 (CH)	C-13, C-15, C-16, C-23
15	2.63 (dd, 6.0, 3.0)	49.7 (CH)	C-2, C-13, C-14, C-16, C-18, C-19, C-23
16	3.47 (br t, 6.9)	50.3 (CH)	C-14
17	6.30 (br s)	—	C-1
18	—	174.2 (C)	
19	1.24 (m)	48.7 (CH_2)	C-15, C-16, C-20, C-21, C-22
20	1.57 (m)	25.1 (CH)	C-19, C-21, C-22
21	0.92 (d, 6.6)	23.4 (CH_3)	C-19, C-20, C-22
22	0.94 (d, 6.6)	21.5 (CH_3)	C-19, C-20, C-21
23	1.28 (d, 7.2)	15.4 (CH_3)	C-13, C-14, C-15

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0.92), one 1,5-dihydroxyhexyl moiety (δ 4.18, 4.08, 4.03, 2.07, 2.03, 1.70, 1.68, 1.37, 1.24), six methine protons (δ 3.47, 3.10, 2.86, 2.63, 2.16, 1.75) and one methyl doublet (δ 1.28). A fused cyclohexane–pyrrolinone unit having a ketone carbonyl moiety at C-1 was established on the basis of the ^{1}H–^{1}H correlation spctroscopy (COSY) correlations of those six methine protons and the amide protons, H-11/H-12, H-13/H-12 and H-14, H-15/H-14 and H-16, H-16/NH-17 and H-14/H_3-23, as well as a ^3J heteronuclear multiple bond correlation (HMBC) of NH-17 with C-1 (δ 64.7) and those of H-11 and H-15 with C-2 (δ 210.4) and C-18 (δ 174.2). According to the ^{1}H and ^{13}C chemical shifts of C-12 (δ 3.10 and δ 54.1) and C-13 (δ 2.86 and δ 54.6) and a coupling constant of 4.5 Hz between H-12 and H-13, a *cis*-epoxide ring was attached at these carbons. The isobutyl group was linked with the pyrrolinone subunit at C-16 (δ 50.3) due to a HMBC correlation of H_2-19 with C-16. The HMBC correlations of the olefinic protons, H-9 with C-11 (δ 49.6) and H-10 with C-8 (δ 75.6) as well as those of H_{ab}-3 with C-2 established an eleven-membered ring. Consequently, **1** had a ring system similar to that of the [11]cytochalasin analogues, for example epoxycytochalasin H,[11] but lacking the methyl groups at C-5, C-7 and C-13 which were derived from L-methionine[12,13] (Fig. 1). The relative configuration of **1** was established by the following nuclear Overhauser effect difference (NOEDIFF) data. Irradiation of H-13 affected signal intensity of H-12 and H_3-23, indicating that they were located at the same side of the molecule. H_3-23 was *cis* to H-16 and *trans* to H-15 on the basis of signal enhancement of only H-16 upon irradiation of H_3-23. Furthermore, signal enhancement of H-11 and H-15 upon irradiation of H-14 supported above conclusion and further revealed the *cis*-relationship between H-11 and H-14. The assigned relative configuration was confirmed by X-ray data (Fig. 2) which further established the relative configuration at C-1, C-4 and C-8. These results also indicated that the cyclohexane and the pyrrolinone subunits were *cis*-fused to each other at C-1 and C-15, and *trans*-fused between the cyclohexane and the cycloundecenone rings at C-1 and C-11. The relative configuration of the cyclohexane and pyrrolinone moieties in **1** was the same as that of the [11]cytochalasins. These proposed that they would have a similar biosynthesis. Compound **1** may be derived from eight acetates and L-leucine instead of L-phenylalanine. Therefore, **1** could possess *S*-configuration at C-16 corresponding to the naturally L-leucine. Ultimately, the 1*R*, 4*S*, 8*S*, 11*R*, 12*S*, 13*R*, 14*S*, 15*R* and 16*S* absolute configuration was proposed for **1**. Therefore, **1** was assigned as a new member of the [11]cytochalasin derivatives.

The crude extract from the culture broth displayed very weak antibacterial activity against SA with the minimum inhibitory concentration (MIC) value of 320 μg/ml and it was inactive against MRSA at the concentration of 1280 μg/ml. All isolated compounds exhibited better activity against both strains than the crude extract with the same MIC value of 200 μg/ml.

Xylarisin (**1**) is a new member of cytochalasin derivatives which contain a highly substituted perhydroisoindole moiety to which, in this case, is linked a macrocarbocylcle ring. The macrocyclic moiety is an octaketide derived from the head-to-tail condensation of eight acetate units to construct an unbranched C_{16}-polyketide moiety.[12] The plausible biogenetic

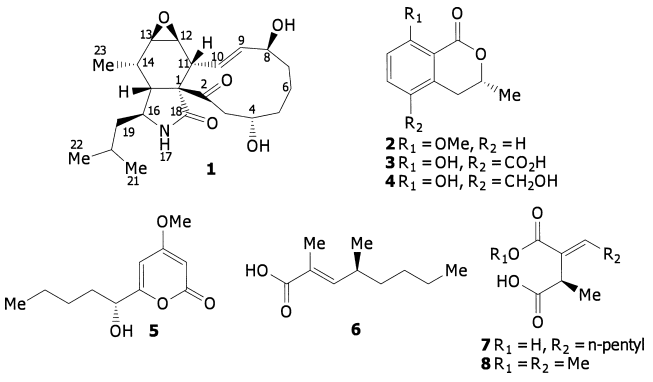

Fig. 1. Structures of Compounds **1**—**8**

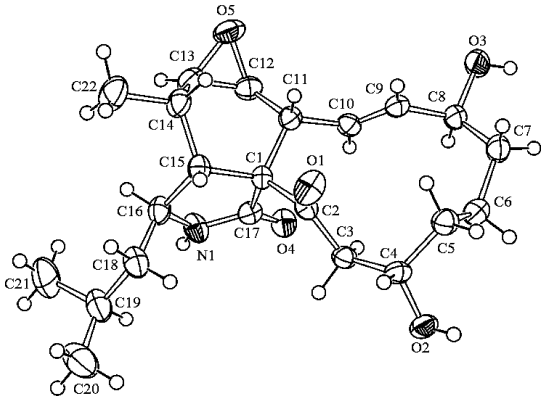

Fig. 2. ORTEP Drawing of Xylarisin (**1**)

pathway would involve the attachment of L-leucine, not L-phenylalanine which was incorporated in all cytochalasins isolated from the genus *Xylaria*,[1,4,14—18] to the C_{16}-polyketide unit. The isolation of RKS-1778, a key intermediate of the [11]cytochalasin group from a soil fungus *Phoma* sp. SNF-1778, provided an important evidence for a Diels-Alder cycloaddition reaction in the biosynthesis.[11]

Experimental

General Procedures Melting point was determined on an Electrothermal 9100 melting point apparatus. Optical rotation was measured on a JASCO P-1020 polarimeter. Ultraviolet (UV) absorption spectrum was recorded in MeOH on a Shimadzu UV-160A spectrophotometer. Infrared spectrum (IR) was obtained on a Perkin-Elmer 783 FTS 165 FT-IR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a 500 MHz Bruker FTNMR Ultra Shield^{TM} spectrometer. Chemical shifts are expressed in δ (ppm) referring to the TMS peak. Mass spectrum was measured on a MAT 95 XL mass spectrometer (Thermofinnigan). TLC was performed on precoated silica gel GF_{254} (Merck) plates (PTLC). Column chromatography (CC) was performed on silica gel (Merck) type 100 (70—230 mesh ASTM), on Sephadex LH-20, or on reverse phase silica gel C-18. Light petroleum had bp 40—60°C.

Organism Collection and Identification The fungus *Xylaria* sp. PSU-F100 was isolated from the gorgonian sea fan (*Annella* sp.) collected near the Similan Islands, Phang-Nga Province by Mr. Sakanan Plathong, the Department of Biology, Faculty of Science, Prince of Songkla University. This fungus was deposited in the BIOTEC Culture Collection as BCC 28783 (GenBank accession number EU714398). It was identified based on analyses of partial LSU rDNA and ITS using the same procedure as that previously reported.[19]

Fermentation and Isolation The flask culture of the marine-derived fungus *Xylaria* sp. PSU-F100 was filtered to separate into the filtrate and wet mycelia. The filtrate (2×6 l) was extracted three times with an equal volume of EtOAc (3×300 ml). The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to yield a dark brown gum

(653.8 mg). The wet mycelia were extracted with 500 ml of MeOH for 2 d. The aqueous MeOH layer was concentrated under reduced pressure. H_2O (50 ml) was added to the extract and the mixture was extracted with EtOAc to afford the crude EtOAc extract as a dark brown gum (1.20 g). The broth extract was chromatographed on Sephadex LH-20 CC with MeOH to afford four fractions (A—D). Fraction B (494.9 mg) was further purified by CC over silica gel with a gradient of MeOH–CH_2Cl_2 to give five subfractions. Subfraction 2 (103.2 mg, eluted with 4—15% MeOH–CH_2Cl_2) was then rechromatographed on CC over silica gel with a gradient of EtOAc–light petroleum to yield **2** (3.6 mg) and **5** (13.5 mg). Subfraction 4 (169.2 mg, eluted with 15% MeOH–CH_2Cl_2) was dissolved with acetone to give colorless crystals of **1** (17.3 mg). After dissolving fraction C (71.3 mg) with $CHCl_3$, a $CHCl_3$-soluble part (42.9 mg) was obtained and then subjected to CC over silica gel eluted with a gradient of EtOAc–light petroleum to give **6** (1.3 mg). Compound **7** (19.9 mg) was obtained when applying fraction D (29.3 mg) to reverse phase CC using 50% MeOH–H_2O as an eluent. The mycelial extract was subjected to CC over Sephadex LH-20 with MeOH to give four fractions. The second fraction (523.4 mg) was purified by CC over silica gel with a gradient of acetone–CH_2Cl_2 to afford **4** (8.6 mg). The last fraction (219.6 mg) was subjected to reverse phase CC with a gradient of MeOH–H_2O to yield **3** (2.8 mg).

Xylarisin (**1**): Colorless crystals. mp 210—212 °C. $[\alpha]_D^{24}$ −88.7 (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 204 (4.45) nm. IR (neat) cm^{-1}: 3377, 3216, 1703, 1693. HR-EI-MS m/z $[M]^+$ 391.2358 (Calcd for $C_{22}H_{33}NO_5$, 391.2359). ^{1}H-NMR ($CDCl_3$, 500 MHz), see Table 1. ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1.

X-Ray Crystallographic Analysis of Xylarisin (1) $C_{22}H_{33}NO_5$ M_r=391.24, orthorhombic, space group $P2_12_12_1$ (no. 24), a=7.9124(3) Å, b=13.9974(4) Å, c=19.2813(6) Å, V=2136.5(1) Å^3, Z=4, D_{calcd}=1.218 g cm^{-3}, μ=0.085 mm^{-1}, 25683 reflections measured, 2931 reflections independent (R_{int}=0.0533), R=0.0506, R_w=0.1065, X-ray diffractions experiments for this compound were carried out on a Bruker Smart Apex CCD diffractometer at 293 K using Mo$K\alpha$ radiation (λ=0.71070 Å). Absorption corrections were done by SADABS. The structure was solved using direct methods and refined with full-matrix least-squares methods based on F^2. Non-hydrogen atoms were refined with anisotropic thermal parameters. The H atoms were located by difference Fourier synthesis and refined with isotropic thermal parameters. The absolute structure of the compound has not been determined based on the X-ray diffraction data; the anomalous scattering power is too small. All calculations were performed using the SHELXTL (v. 6.14) package. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (deposit No. CCDC 731711). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44–(0)223–336033 or e-mail: deposit@ccdc.cam.ac.uk).

Biological Assays Antibacterial activity was evaluated against SA and MRSA, using the agar microdilution method.[20] MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Vancomycin, a positive control drug, exhibited the MIC value of 1 μg/ml. Growth controls were performed on the agar containing DMSO.

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