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Amide, cyclohexenone, and cyclohexenone–sordaricin derivatives from the endophytic fungus *Xylaria plebeja* PSU-G30

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ABSTRACT

Six new compounds, two cyclohexenones, named xylariacyclones A (**1**) and B (**2**), three cyclohexenone–sordaricin derivatives, named xylarinonericins A–C (**3**–**5**), and one amide derivative, named xylariamide (**6**), together with 11 known compounds were isolated from the broth extract of the endophytic fungus *Xylaria plebeja* PSU-G30. The structures were elucidated by analyses of NMR spectroscopic data and chemical methods. Compounds **3**–**5** are novel and unusual sordaricin derivatives with an ester moiety at C-6 of the sordaricin skeleton. In addition, compound **5** has a unique feature with an ester unit instead of an ether group at C-19. They were evaluated for antifungal activity against *Candida albicans* ATCC90028 and *Cryptococcus neoformans* ATCC90113.

1. Introduction

The endophytic fungi in the genus *Xylaria* produce many types of secondary metabolites,[1–3] for example, sordaricins,[4] lactones,[5] cytochalasins,[6] and succinic acid derivatives.[7] Some of them show interesting biological activities, such as the antifungal sordaricin,[8] antiplasmodial (+)-phomalactone,[9] cytotoxic (+)-mycoepoxydiene, and deacetylmycoepoxydiene.[10] Our previous investigation on endophytic fungi isolated from *Garcinia hombroniana* revealed that they are a rich source of new and bioactive secondary metabolites with diverse structures.[10–13] In this paper, we describe the isolation and structure determination of six new (**1**–**6**) and 11 known (**7**–**17**) secondary metabolites from the endophytic fungus *Xylaria plebeja* PSU-G30, which was isolated from a branch of *G. hombroniana*, collected from Songkhla province, Thailand. The cyclohexenone (**1** and **8**) and sodaricin (**3**–**5**) derivatives were tested for antifungal activity against *Candida albicans* ATCC90028 and *Cryptococcus neoformans* ATCC90113. To our best knowledge, this is the first report on secondary metabolites produced by *X. plebeja*.

2. Results and discussion

Purification of the broth extract of *X. plebeja* PSU-G30 using chromatographic techniques afforded six new compounds: two cyclohexenone derivatives, named xylariacyclones A (**1**) and B (**2**), three cyclohexenone–sordaricin derivatives, named xylarinonericins A–C (**3**–**5**), and one amide derivative, named xylariamide (**6**), along with 11 known compounds: *N*-isovaleroyl isoamylamine (**7**),[14] (4*R*,5*S*,6*R*)-4,5,6-trihydroxy-3-methoxy-5-methylcyclohex-2-en-1-one (**8**),[15] 5-hydroxy-3-methoxy-6-oxo-2-decanoic acid δ-lactone (**9**),[16] PC-2 (**10**),[17] pestalotin (**11**),[18] LL-P880γ (**12**),[19] xylaranol B (**13**),[20] 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**14**),[21] (3*R*,4*R*)-4-hydroxymellein (**15**),[22] (3*R*,4*S*)-4-hydroxymellein (**16**),[22] and (3*R*)-mellein (**17**)[23] (Fig. 1). Their structures were elucidated on the basis of IR, UV, NMR, and MS data.

For known compounds, the structures were confirmed by comparison of the ^{1}H and ^{13}C NMR data with those previously reported. Their relative configurations were assigned on the basis of NOEDIFF data whereas the absolute configurations were determined by comparison of their optical rotations with those previously reported in the literature. The relative configuration of the cyclohexenone unit in **1** was established by NOEDIFF data of its

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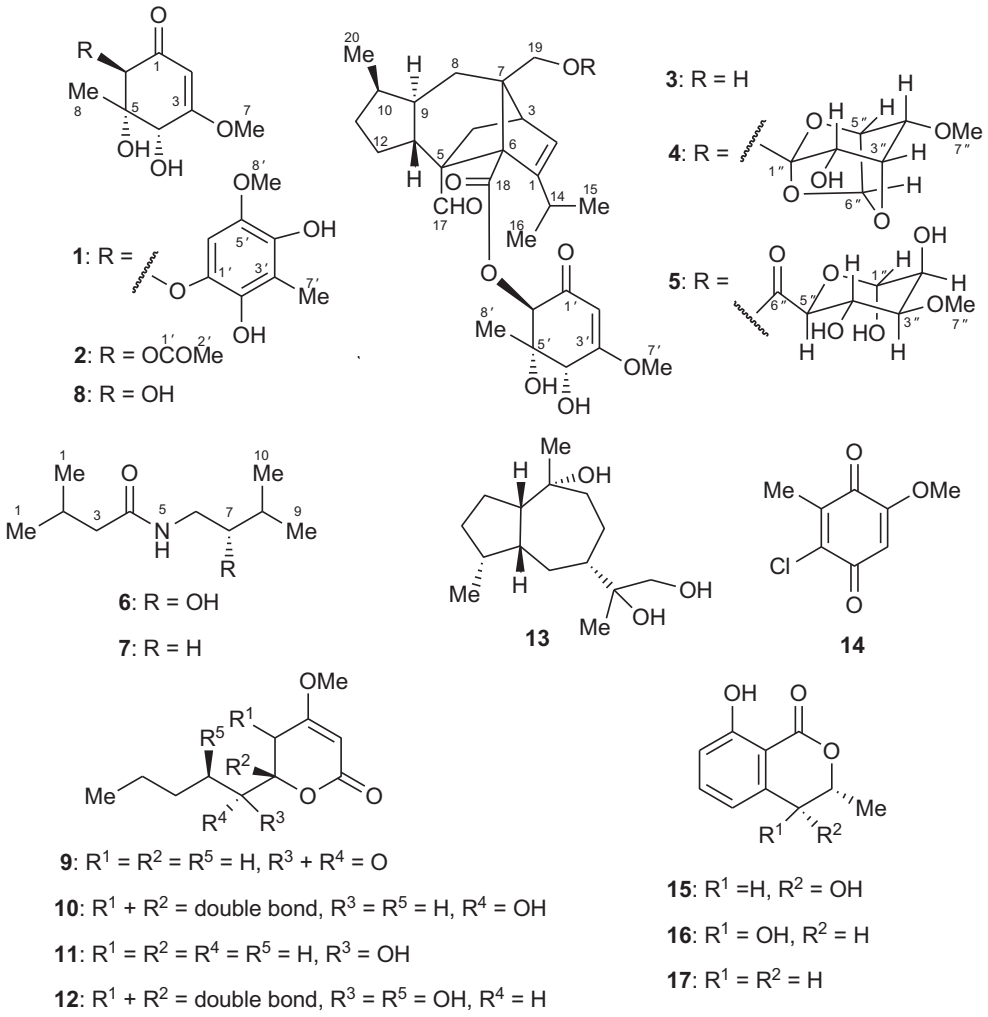

Fig. 1. Structures of compounds **1**–**17** isolated from *Xylaria plebeja* PSU-G30.

acetonide derivative (**1a**) while that of **2**–**5** was assigned by comparison of their NOEDIFF data with those of **1**. Its absolute configuration was established according to the known absolute configuration of the co-metabolite **8**. For **3**–**5**, the relative configuration of the sordaricin moiety was assigned by NOEDIFF data and the absolute configuration was established by comparison of the optical rotation of sordaricin obtained from hydrolysis of **5** with that of natural sordaricin. The absolute configuration of **6** was determined by comparing its optical rotation with that of a structurally related compound.

Xylariacyclone A (**1**) with the molecular formula $C_{16}H_{20}O_8$ by HREIMS was obtained as a colorless gum. The UV spectrum showed maximum absorption bands at 221, 248, and 287 nm. The absorption band at 248 nm revealed the presence of an α,β-unsaturated ketone chromophore.[15] This conclusion was supported by an IR absorption band at 1668 cm^{-1}. The ^1H NMR spectroscopic data (Table 1) consisted of signals similar to those of **8**, including one olefinic proton (δ_H 5.43, d, J=1.5 Hz), two oxygenated methine protons (δ_H 4.44, s and 4.08, d, J=1.5 Hz), methoxyl protons (δ_H 3.80, s), and methyl protons (δ_H 1.36, s). The presence of a 6-oxygenated-4,5-dihydroxy-3-methoxy-5-methylcyclohex-2-en-1-one was supported by the HMBC correlations shown in Fig. 2. In addition, **1** displayed signals for one aromatic proton of a pentasubstituted aromatic ring (δ_H 6.88, s), two phenolic hydroxy protons (δ_H 8.84 and 5.80, each s), one methoxyl group (δ_H 3.81, s), and one methyl group (δ_H 2.18, s). The hydroxyl groups were assigned at C-2′ (δ_C 146.3) and C-4′ (δ_C 145.1) on the basis of the ^{13}C chemical shifts of C-2′ and C-4′ as well as the HMBC correlations from 2′-OH (δ_H 8.84) to C-1′ (δ_C 143.3), C-2′ and C-3′ (δ_C 115.9) and those from 4′-OH (δ_H 5.80) to C-3′, C-4′ and C-5′ (δ_C 142.7) (Fig. 2). The methyl protons, H_3-7′ (δ_H 2.18), displayed the HMBC correlations with C-2′, C-3′ and C-4′, thus connecting the methyl group at C-3′. The methoxyl group at δ_H 3.81 (H_3-8′) was attached at C-5′ on the basis of the ^3J HMBC correlation of H_3-8′ and C-5′. Consequently, the aromatic proton (δ_H 6.88) was attributed to H-6′. This assignment was supported by its HMBC correlations with C-1′, C-2′, C-4′, and C-5′ as well as signal enhancement of H_3-8′ upon irradiation of H-6′. These results together with the chemical shift of C-1′ established a 1′-oxy-2′,4′-dihydroxy-5′-methoxy-3′-methylbenzene moiety. The oxymethine proton (H-6, δ_H 4.44) of the cyclohexenoyl unit displayed the ^3J HMBC correlation with C-1′, thus connecting this unit with C-1′ of the pentasubstituted aromatic ring by forming an ether linkage between C-1′ and C-6. The relative configuration of **1** was identical to that of the co-metabolite **8** according to the following NOEDIFF data of an acetonide derivative[15] of **1** (**1a**) (Fig. 2). Irradiation of H-4 (δ_H 4.51) affected signal intensity of H_3-8 (δ_H 1.47) and H_3-2″ (δ_H 1.55) while signal enhancement of H_3-3″ (δ_H 1.61) was observed upon irradiation of H-6 (δ_H 4.40) (Fig. 2). These results indicated the *cis*-relationship of H-4, H_3-8 and the aromatic ether unit at C-6. Because **1** and **8** were co-metabolites and both compounds displayed the same sign of the optical rotations, the absolute configurations at C-4, C-5, and C-6 in **1** were proposed to be *R*, *S*, and *R*,

respectively, identical to those of **8**. Thus, xylariacyclone A had the structure **1**.

Table 1
^{1}H and ^{13}C NMR data for **1** in $CDCl_3$+CD_3OD and **1a** and **2** in $CDCl_3$

Position	**1**		**1a**		**2**	
	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	200.4, C		198.0, C		190.8, C	
2	104.5, CH	5.43, d (1.5)	102.3, CH	5.52, s	101.2, CH	5.40, s
3	178.4, C		169.0, C		173.0, C	
4	78.7, CH	4.08, d (1.5)	79.7, CH	4.51, s	74.9, CH	4.16, s
5	77.9, C		83.0, C		73.5, C	
6	92.4, CH	4.44, s	90.1, CH	4.40, s	76.4, CH	5.70, s
7	60.6, CH_3	3.80, s	57.3, CH_3	3.83, s	56.8, CH_3	3.79, s
8	22.6, CH_3	1.36, s	18.5, CH_3	1.47, s	19.4, CH_3	1.28, s
1′	143.3, C		139.0, C		171.2, C	
2′	146.3, C		141.3, C		20.8, CH_3	2.26, s
2′-OH		8.84, s		8.62, s		
3′	115.9, C		111.9, C			
4′	145.1, C		142.6, C			
4′-OH		5.80, s		5.50, s		
5′	142.7, C		138.5, C			
6′	107.5, CH	6.88, s	103.6, CH	6.79, s		
7′	12.4, CH_3	2.18, s	14.3, CH_3	2.18, s		
8′	60.7, CH_3	3.81, s	56.2, CH_3	3.79, s		
1″			111.1, C			
2″			26.6, CH_3	1.55, s		
3″			27.9, CH_3	1.61, s		

cyclohexenone moiety, identical to that in **1** and **2**, which had three degrees of unsaturation. Furthermore, it revealed the replacement of the acetoxyl signal in **2** with signals for a sordaricin moiety in **3**.[24] The sodaricin unit with seven degrees of unsaturation showed characteristic signals for one aldehyde proton (δ_H 9.71, s), one olefinic proton (δ_H 6.04, dd, J=3.6 and 1.2 Hz), five sets of non-equivalent methylene protons [δ_H 3.97/3.42, each 1H, d, J=11.7 Hz; 2.02 (m, 1H)/1.64 (dd, J=13.5 and 6.3 Hz, 1H); 2.00/1.25, each 1H, m; 1.97/1.17, each 1H, m; 1.92/0.94, each 1H, m], five methine protons [δ_H 2.60 (td, J=11.4 and 5.4 Hz, 1H); 2.47 (br t, J=3.6 Hz, 1H); 2.39 (heptd, J=6.6 and 1.2 Hz, 1H); 2.10 (m, 1H); 1.73 (m, 1H)], and three methyl groups [δ_H 0.99 (d, J=6.6 Hz, 3H), 0.87 (d, J=6.6 Hz, 3H) and 0.86 (d, J=6.9 Hz, 3H)]. Its ^{13}C NMR spectrum (Table 2), apart from carbon signals of the cyclohexenone unit, displayed one aldehyde carbonyl (δ_C 204.6), one ester carbonyl (δ_C 173.4), four quaternary (δ_C 148.6, 73.1, 67.8, and 59.2), six methine (δ_C 130.7, 47.4, 41.4, 40.9, 31.6 and 27.7), five methylene (δ_C 67.0, 32.1, 30.2, 28.0, and 26.3), and three methyl (δ_C 22.9, 21.4, and 17.2) carbons. The sordaricin moiety was established by the ^{1}H–^{1}H COSY and HMBC correlations (Fig. 3). The relative configuration of the sordaricin unit was assigned by the following NOEDIFF data (Fig. 3). Signals of H-17 (δ_H 9.71) and H_3-20 (δ_H 0.86) were enhanced when H-13 (δ_H 2.60) was irradiated. Moreover, irradiation of H-17 enhanced signal intensity of H-13 and H-14 (δ_H 2.39). These results established a *trans*-fused cyclopentane unit and *cis*-relationship between H-13 and H_3-20 as well as between the aldehyde unit and the isopropyl moiety. The ^3J HMBC correlation from the oxymethine proton, H-6′ (δ_H 5.75), of the cyclohexenoyl unit to C-18 (δ_C 173.4) of the sordaricin unit

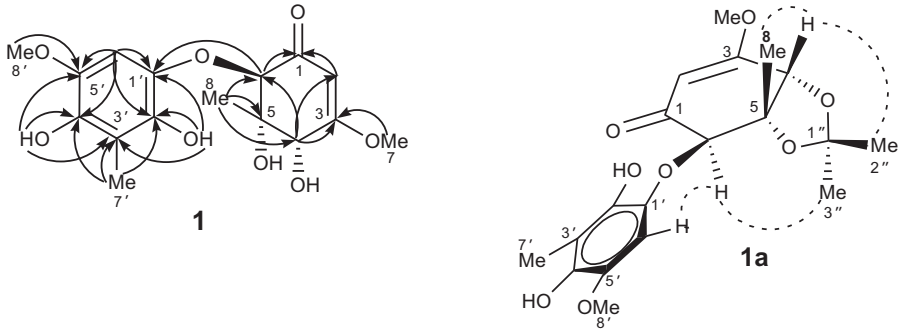

Fig. 2. Selected HMBC (⌒) data of **1** and NOEDIFF (⋯) data of **1a**.

Xylariacyclone B (**2**) was obtained as a colorless gum with the molecular formula $C_8H_{12}O_5$. The UV (247 nm) and IR (1667 cm^{-1}) spectra indicated the presence of an α,β-unsaturated ketone. The IR spectrum exhibited an additional absorption band at 1737 cm^{-1} for an ester carbonyl group. The ^{1}H NMR spectroscopic data (Table 1) were similar to those of **1** except for the replacement of signals for the pentasubstituted aromatic ring with signal for an acetoxyl group (δ_H 2.26, s) in **2**. The HMBC correlations of H_3-2′ (δ_H 2.26) and H-6 (δ_H 5.70) with C-1′ (δ_C 171.2) established the attachment of an acetoxyl group at C-6 (δ_C 76.4). The absolute configurations at all chiral carbons in **2** were proposed to be identical to **1** on the basis of similar optical rotation and identical NOEDIFF data. Therefore, the structure **2** was assigned for xylariacyclone B.

Xylarinonericin A (**3**) was obtained as a colorless gum with the molecular formula $C_{28}H_{38}O_8$ by HRESIMS representing ten degrees of unsaturation. The UV spectrum displayed absorption bands at 247, 266, and 330 nm. The IR spectrum exhibited absorption bands at 3401 cm^{-1} for hydroxyl and 1712 and 1674 cm^{-1} for unconjugated and conjugated carbonyl groups, respectively. The ^{1}H NMR spectrum (Table 2) indicated the presence of the connected the cyclohexenoyl unit with the sordaricin moiety by forming an ester linkage between C-18 and C-6′. The relative configuration of the cyclohexenone unit was identical to that of **1** and **2** according to their identical NOEDIFF data. Irradiation of H_3-8′ affected signal intensity of H-14 and H-17, indicating their close proximity. Based on the absolute configuration of the cyclohexenone unit, the absolute configuration in the sordaricin moiety was proposed as 3*R*, 5*S*, 6*R*, 7*S*, 9*R*, 10*R*, and 13*R*, which were identical to sordaricin.[24] Consequently, xylarinonericin A (**3**) was identified as a new cyclohexenone–sordaricin derivative.

Xylarinonericin B (**4**) was obtained as a colorless gum and had the molecular formula $C_{35}H_{46}O_{13}$ by HRESIMS. The UV and IR spectra displayed absorption bands similar to those of **3**. Their ^{1}H NMR spectroscopic data (Table 2) were similar except for additional signals for a monomethyl ether derivative of guluronic acid moiety[25] [δ_H 5.67 (d, J=2.5 Hz, 1H), 4.55 (td, J=3.5 and 2.5 Hz, 1H), 4.33 (m, 1H), 3.73 (d, J=8.5 Hz, 1H), 3.65 (t, J=4.0 Hz, 1H), 3.49 (s, 3H) and 2.47 (d, J=8.5 Hz, 1H)] in **4**. The *O*-methylated guluronic acid unit was established based on the following ^{1}H–^{1}H COSY correlations: H-3″ (δ_H 4.33)/H-2″ (δ_H 3.73) and H-4″ (δ_H 3.65) and H-5″ (δ_H 4.55)/

Table 2
^{1}H and ^{13}C NMR data for **3** and **4** in $CDCl_3$ and **5** in CD_3OD

Position	**3**		**4**		**5**	
	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)	δ_C, type	δ_H, mult (*J* in Hz)
1	148.6, C		147.6, C		148.5, C	
2	130.7, CH	6.04, dd (3.6, 1.2)	131.4, CH	6.20, br d (3.0)	130.6, CH	6.13, dd (3.9, 1.2)
3	47.4, CH	2.47, br t (3.6)	46.0, CH	2.82, t (4.0)	46.1, CH	2.81, br t (3.9)
4	30.2, CH_2	a: 1.97, m	29.6, CH_2	a: 1.93, dd (12.5, 4.5)	29.0, CH_2	a: 1.92, m
		b: 1.17, m		b: 1.31, d (12.5)		b: 1.35, m
5	59.2, C		58.9, C		58.4, C	
6	73.1, C		72.0, C		71.9, C	
7	67.8, C		65.9, C		65.6, C	
8	28.0, CH_2	a: 2.02, m	28.8, CH_2	a: 2.00, dd (13.5, 5.5)	28.7, CH_2	a: 2.08, m
		b: 1.64, dd (13.5, 6.3)		b: 1.77, d (13.5)		b: 1.32, m
9	41.4, CH	1.73, m	41.5, CH	1.72, m	41.2, CH	1.83, m
10	31.6, CH	2.10, m	31.4, CH	2.09, m	31.3, CH	2.08, m
11	32.1, CH_2	a: 2.00, m	32.2, CH_2	a: 2.03, m	31.8, CH_2	a: 2.07, m
		b: 1.25, m		b: 1.35, m		b: 1.32, m
12	26.3, CH_2	a: 1.92, m	26.2, CH_2	a: 1.76, m	25.8, CH_2	a: 1.91, m
		b: 0.94, m		b: 0.99, m		b: 0.98, m
13	40.9, CH	2.60, td (11.4, 5.4)	40.9, CH	2.72, td (12.0, 7.0)	40.6, CH	2.88, m
14	27.7, CH	2.39, heptd (6.6, 1.2)	27.7, CH	2.41, hept (7.0)	27.2, CH	2.58, m
15	21.4, CH_3	0.99, d (6.6)	21.6, CH_3	1.04, d (7.0)	22.5, CH_3	1.03, d (6.6)
16	22.9, CH_3	0.87, d (6.6)	22.9, CH_3	0.93, d (7.0)	20.4, CH_3	0.96, d (6.6)
17	204.6, CH	9.71, s	204.8, CH	9.81, s	204.6, CH	9.76, s
18	173.4, C		171.6, C		171.0, C	
19	67.0, CH_2	a: 3.97, d (11.7)	67.1, CH_2	a: 4.10, d (10.0)	70.2, CH_2	a: 4.50 (d, 9.6)
		b: 3.42, d (11.7)		b: 4.03, d (10.0)		b: 4.34 (d, 9.6)
20	17.2, CH_3	0.86, d (6.9)	17.2, CH_3	0.90, d (7.0)	16.3, CH_3	0.89, d (6.6)
1′	190.0, C		190.3, C		191.8, C	
2′	101.0, CH	5.35, d (0.9)	101.1, CH	5.41, s	99.6, CH	5.42, d (1.2)
3′	173.0, C		172.7, C		176.2, C	
4′	75.1, CH	4.08, br s	75.1, CH	4.14, s	75.0, CH	3.99, d (1.2)
4′-OH				3.11, s		
5′	73.1, C		73.1, C		72.7, C	
5′-OH				2.90, s		
6′	77.6, CH	5.75, s	77.1, CH	5.79, s	77.2, CH	5.80, s
7′	56.8, CH_3	3.71, s	56.8, CH_3	3.77, s	56.2, CH_3	3.81, s
8′	19.7, CH_3	1.20, s	19.9, CH_3	1.27, s	18.7, CH_3	1.20, s
1″			118.6, C		94.6, CH	5.15, d (3.0)
2″			66.8, CH	3.73, d (8.5)	67.5, CH	3.93, t (3.0)
2″-OH				2.47, d (8.5)		
3″			77.9, CH	4.33, m	80.5, CH	3.46, dd (8.4, 3.0)
4″			80.0, CH	3.65, t (3.5)	67.4, CH	4.04, t (8.4)
5″			74.4, CH	4.55, td (3.5, 2.5)	73.0, CH	4.22, d (8.4)
6″			100.2, CH	5.67, br d (2.5)	170.3, C	
7″			57.7, CH_3	3.49, s	56.5, CH_3	3.50, s

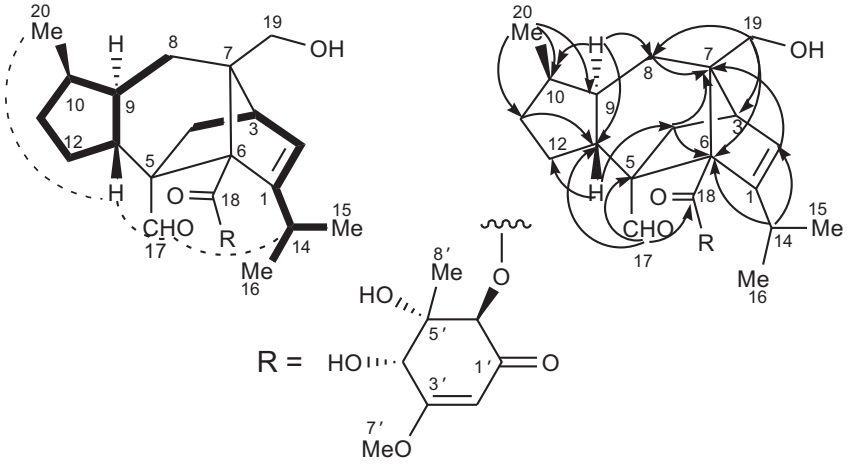

Fig. 3. Selected COSY (—), NOEDIFF (⋯), and HMBC (⌒) data of **3**.

H-4″ and H-6″ (δ_H 5.67). The HMBC correlations of H-5″ and H-6″ with C-1″ (δ_C 118.6) established two ether linkages between C-1″/C-5″ (δ_C 74.4) and C-1″/C-6″ (δ_C 100.2). The oxymethine proton, H-3″, displayed the HMBC cross peak with C-6″ constructed the third ether linkage between C-3″ (δ_C 77.9)/C-6″. The methoxyl and hydroxyl groups at δ_H 3.49 and 2.47 were attached at C-4″ and C-2″, respectively, on the basis of their ^{3}J HMBC correlations. These results and the chemical shifts of C-1″ and C-6″ established the

tricyclic uronic acid moiety. The nonequivalent oxymethylene protons (H_2-19, δ_H 4.10 and 4.03) of the sordaricin unit displayed the ^3J HMBC correlation with C-1″ of the tricyclic uronic acid moiety, thus connecting these two units by forming an ether linkage between C-19 (δ_C 67.1) and C-1″. The relative configurations of the sordaricin and cyclohexenoyl units in **4** were identical to those in **3** according to identical NOEDIFF data. The relative configuration of the tricyclic uronic acid unit was determined by the coupling constants and the following NOEDIFF data. Signal of H-4″ was enhanced upon irradiation of H-2″, indicating their axial arrangement. Consequently, both 2″-OH and 4″-OCH_3 were at equatorial positions.[25] As H-4″ was coupled with H-3″ and H-5″ with an identical small coupling constant of 3.5 Hz, both H-3″ and H-5″ were located at equatorial positions. In addition, H-5″ and H-6″ were located at the same side of the molecule as irradiation of H-5″ affected signal intensity of H-6″. The NOEDIFF data obtained were inadequate to relate the stereochemistry of the cyclohexenone–sordaricin unit and the sugar moiety. The absolute configuration of the sugar part was not determined due to the small amount of **4** that remained. Thus, the structure **4** was assigned for xylarinonericin B.

Xylarinonericin C (**5**) with the molecular formula $C_{35}H_{48}O_{14}$ by HRESIMS, was obtained as a white solid and melted at 147.5–149.0 °C. The UV and IR spectra displayed absorption bands similar to those of **3**. Their ^{1}H NMR spectroscopic data (Table 2) were similar except for additional signals for a monomethylated sugar moiety [δ_H 5.15 (d, J=3.0 Hz, 1H), 4.22 (d, J=8.4 Hz, 1H), 4.04 (t, J=8.4 Hz, 1H), 3.93 (t, J=3.0 Hz, 1H), 3.50 (s, 3H), and 3.46 (dd, J=8.4 and 3.0 Hz, 1H)] in **5**. The sugar unit was established based on the following ^{1}H–^{1}H COSY correlations: H-2″ (δ_H 3.93)/H-1″ (δ_H 5.15) and H-3″ (δ_H 3.46) and H-4″ (δ_H 4.04)/H-5″ (δ_H 4.22) and H-3″. The HMBC correlations of H-5″ with C-1″ (δ_C 94.6) formed an ether linkage between C-1″ and C-5″ (δ_C 73.0) while that of H-5″ with C-6″ (δ_C 170.3) attached an ester carbonyl group at C-5″. The methoxyl group at δ_H 3.50 was attached at C-3″ (δ_C 80.5) on the basis of its ^3J HMBC correlation. The chemical shifts of C-1″, C-2″ (δ_C 67.5), and C-4″ (δ_C 67.4) indicated the attachment of the hydroxyl groups at these carbons. The relative configuration of the sugar unit was determined by the NOEDIFF data and the coupling constants observed in the ^{1}H NMR spectrum. H-4″ was coupled with H-3″ and H-5″ with an identical coupling constant of 8.4 Hz, indicating that these protons were at axial positions. In addition, signal intensity of H-2″, but not H-1″, was enhanced upon irradiation of H-3″. These results indicated that both H-1″ and H-2″ were at equatorial positions. The nonequivalent oxymethylene protons (H_2-19, δ_H 4.50 and 4.34) of the sordaricin unit displayed the ^3J HMBC correlation with C-6″ of the sugar unit, thus forming an ester linkage between C-19 (δ_C 70.2) of the sordaricin unit and C-1″ of the sugar moiety. The absolute configurations of the cyclohexenone and sordaricin were proposed to be identical to those of **3** on the basis of the NOEDIFF data. The similarity of the optical rotation of sordaricin obtained from **5** by hydrolysis with LiOH, $[\alpha]_D^{27}$ −70.1 (*c* 0.10, MeOH), to that of natural sordaricin, $[\alpha]_D^{20}$ −58.4 (*c* 0.19, MeOH),[24] as well as their identical TLC chromatogram confirmed the identical absolute configuration of the sordaricin unit in **3**–**5** to that of natural sordaricin. Unfortunately, the sugar residue was not obtained. Attempts to obtain a single crystal of **5** for X-ray analysis were unsuccessful. Thus, the absolute configuration of the sugar moiety in **5** remained unidentified. Therefore, xylarinonericin C had the structure **5**.

Xylariamide (**6**) was obtained as a colorless gum and had the molecular formula $C_{10}H_{21}NO_2$ by FABMS for $[M+H]^+$. Hydroxyl and amino absorption bands were found at 3313 cm^{-1}, while an amide carbonyl absorption band was found at 1644 cm^{-1} in the IR spectrum. The ^{1}H NMR spectrum (Table 3) contained signals for a 1-amino-3-methyl-2-substituted butyl unit [δ_H 5.83 (br s, 1H), 3.45 (ddd, J=13.8, 6.6, 2.7 Hz, 1H), 3.35 (ddd, J=8.7, 6.3, 2.7 Hz, 1H), 3.10 (ddd, J=13.8, 8.7, 4.5 Hz, 1H), 1.61 (m, 1H), 0.91 (d, J=6.9 Hz, 3H), and 0.87 (d, J=6.9 Hz, 3H)] and a 3-methylbutanoyl unit [δ_H 2.05 (m, 1H), 2.01 (d, J=7.5 Hz, 2H), and 0.89 (d, J=6.3 Hz, 6H)]. The ^{13}C NMR spectrum (Table 3) showed one amide carbonyl (δ_C 173.6), three methine (δ_C 77.2, 32.2, and 26.2), two methylene (δ_C 46.1 and 43.6), and four methyl (three resonances for four carbons, δ_C 22.4x2, 18.6, and 17.7) carbons. The ^{1}H and ^{13}C NMR spectroscopic data (Table 3) were similar to those of **7** except for the replacement of signal for the chemically equivalent methylene protons (δ_H 1.32) in **7** with that for an oxymethine proton (δ_H 3.35) in **6**. Signal of an oxymethine carbon at δ_C 77.2 in the ^{13}C NMR spectrum supported above conclusion. The oxymethine proton was assigned as H-7 on the basis of its ^{1}H–^{1}H COSY correlations with H_{ab}-6 (δ_H 3.45 and 3.10) and H-8 (δ_H 1.61) as well as its HMBC correlations with C-6 (δ_C 43.6), C-8 (δ_C 32.2), C-9 (δ_C 18.6), and C-10 (δ_C 17.7). This compound was proposed to possess *S* configuration at C-7, identical to (+) *N*-(*t*-Boc)-1-aminopropan-2-ol according to their similar optical rotation, $[\alpha]_D^{26}$ +26.6 (*c* 1.00, CH_2Cl_2) of **6** and $[\alpha]_D^{20}$ +27.5 (*c* 1.00, CH_2Cl_2) of (+) *N*-(*t*-Boc)-1-aminopropan-2-ol.[26] Thus, xylariamide possessed the structure **6**.

Table 3
^{1}H, ^{13}C NMR and HMBC data for **6** in $CDCl_3$

Position	δ_C, type	δ_H, mult (*J* in Hz)	HMBC
1	22.4, CH_3	0.89, d (6.3)	C-2, C-3
2	26.2, CH	2.05, m	C-1, C-3, C-4
3	46.1, CH_2	2.01, d (7.5)	C-1, C-2, C-4
4	173.6, C		
NH-5		5.83, br s	
6	43.6, CH_2	a: 3.45, ddd (13.8, 6.6, 2.7)	C-4, C-7, C-8
		b: 3.10, ddd (13.8, 8.7, 4.5)	C-4, C-7, C-8
7	77.2, CH	3.35, ddd (8.7, 6.3, 2.7)	C-6, C-8, C-9, C-10
8	32.2, CH	1.61, m	C-6, C-7, C-9, C-10
9	18.6, CH_3	0.87, d (6.9)	C-7, C-8, C-10
10	17.7, CH_3	0.91, d (6.9)	C-7, C-8, C-9

Sordaricin derivatives have been reported to display interesting antifungal activity against a wide range of fungal pathogens, especially *C. albicans* and *C. neoformans*.[27–33] Sordaricin, the diterpene aglycone of sordarins, has been reported by our research group to exhibit moderate activity against *C. albicans* ATCC90028.[8] Therefore, the isolated sordaricin derivatives (**3**–**5**) as well as the cyclohexenones (**1** and **8**) were tested against *C. albicans* ATCC90028 and *C. neoformans* ATCC90113. Unfortunately, all of them were inactive against both fungal strains at the concentration of 200 μg/mL. The lack of activity of **3** suggested that the inclusion of a cyclohexenone moiety at C-6 abolishes the antifungal activity of sordaricin. This result would be consistent with the finding reported by Schneider et al.,[25] who observed that the modification of xylarin to incorporate a methyl-ester group at the same C-6 position decreased the antifungal activity of the parent compound.

3. Conclusion

Six new secondary metabolites, including three sordaricin derivatives (**3**–**5**), were isolated from the broth extract of the endophytic fungus *X. plebeja* PSU-G30, which was isolated from a branch of *G. hombroniana*. Sordaricin derivatives have been isolated from a large number of species and strains of the genus *Xylaria*.[4] Structurally, all the sordaricin derivatives reported to date have a carboxylic moiety and an ether substituent at C-6 and C-19, respectively.[25,27–31,33–41] The isolated sordaricin derivatives (**3**–**5**) from the fungus PSU-G30 carried an ester functionality instead of a carboxylic acid moiety at C-6. In addition, compound **5** possessed an ester substituent at C-19. Thus, compounds **3**–**5** are novel and unusual sordaricin derivatives. Based on the absolute configurations of the cyclohexenone unit in **3**–**5**, compounds **2** and **8** might be precursors for the synthesis of these compounds.

4. Experimental section

4.1. General experimental procedures

Infrared (IR) spectra were recorded on a Perkin–Elmer 783 FTS 165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a 300 or a 500 MHz Bruker FTNMR Ultra Shield spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra, EIMS and HREIMS spectra, were obtained from a MAT 95 XL Mass Spectrometer (Thermo Finnigan) whereas ESITOF MS was determined using a liquid chromatogram–mass spectrometer LCT (micromass). Thin-layer chromatography (TLC) and precoated TLC (PTLC) were performed on silica gel GF_{256} (Merck). Column Chromatography (CC) was carried out on Sephadex LH-20, silica gel (Merck) type 60 (230–400 mesh ASTM) or type 100 (70–230 mesh ASTM), or reverse phase C_{18} silica gel.

4.2. Fungal material

The endophytic fungus PSU-G30 was isolated from a branch of *G. hombroniana* from Songkhla province, Thailand. It was deposited as BCC 35877 at Biotec Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. This endophytic fungus did not produce any conidia or spores. Therefore, it was identified based on the analysis of the DNA sequences of the internal transcribed spacer (ITS1-5.8S-ITS2) regions of its ribosomal RNA gene. Its ITS sequence (GenBank accession number JQ623492) matched with *Xylaria plebeja* (GU324740) and *Xylaria* sp. (DQ480344) sequences from GenBank with sequence identity of 99.2%. The endophytic fungus was then identified to be *X. plebeja*.

4.3. Fermentation, extraction, and isolation

The fungus PSU-G30 was grown on potato dextrose agar (PDA) at 25 °C for 5 days. Three pieces (0.5×0.5 cm^2) of mycelial agar plugs were inoculated into 500 mL Erlenmeyer flasks containing 300 mL potato dextrose broth (PDB) at room temperature for 3 weeks. The culture (15 L) was filtered to give the filtrate and mycelia. The filtrate was extracted with EtOAc to afford a broth extract (1.73 g) as a dark brown gum after evaporation of the EtOAc extract to dryness. The crude extract was fractionated by CC over Sephadex LH-20 to give four fractions (A–D). Fraction B (1.24 g) was purified by CC over silica gel with a gradient of methanol in dichloromethane to yield eight subfractions (B1–B8). Fraction B2 (180.6 mg) was further separated by CC over silica gel using a gradient of ethyl acetate in petroleum ether to afford 10 subfractions (B2A–B2J). Subfraction B2C (43.4 mg) was further separated by CC over silica gel using a gradient of ethyl acetate in dichloromethane as a mobile phase to afford three subfractions. The second subfraction (28.0 mg) was purified twice using the same procedure as subfraction B2C to give **7** (10.1 mg). Subfraction B2E (14.2 mg) was purified using the same procedure as subfraction B2C to give five subfractions. The second subfraction contained **9** (3.4 mg). Subfraction B2G (13.7 mg) was further separated by CC over silica gel using a gradient of ethyl acetate in dichloromethane as a mobile phase to afford three subfractions. The second subfraction (9.4 mg) was further purified by PTLC with 10% ethyl acetate in dichloromethane as a mobile phase (4 runs) to give **10** (3.0 mg). Subfraction B2I (28.6 mg) was purified using the same procedure as subfraction B2G to afford four subfractions. The second subfraction contained **11** (11.3 mg). Subfraction B3 (10.6 mg) was purified by PTLC using 7% ethyl acetate in dichloromethane as a mobile phase (9 runs) to give **6** (2.4 mg). Subfraction B4 (69.7 mg) was purified by CC over reverse phase C_{18} silica gel using a gradient of acetone in water to give six subfractions (B41–B46). Subfraction B46 (35.3 mg) was purified using the same procedure as subfraction B4 to afford three subfractions. The second subfraction (12.9 mg) was further separated by CC over silica gel using a gradient of ethyl acetate in dichloromethane to afford two subfractions. The first subfraction (6.2 mg) was further purified by PTLC with 30% ethyl acetate in dichloromethane as a mobile phase (2 runs) to give **3** (3.4 mg) and **4** (2.8 mg). Subfraction B5 (97.9 mg) was purified by CC over reverse phase C_{18} silica gel using a gradient of methanol in water to give five subfractions (B51–B55). Subfraction B53 (7.2 mg) was further purified by PTLC with 20% ethyl acetate in dichloromethane as a mobile phase (4 runs) to give **2** (1.3 mg). Subfraction B55 (11.0 mg) was subjected to CC over Sephadex LH-20 using a mixture of methanol, dichloromethane, and petroleum ether in the ratio of 2:2:1 to afford three subfractions. The second subfraction (9.6 mg) was purified using the same procedure as subfraction B53 to afford **12** (1.4 mg). Subfraction B6 (140.5 mg) was purified using the same procedure as subfraction B5 to afford six subfractions (B61–B66). Subfraction B65 (74.0 mg) was purified using the same procedure as subfraction B6 to afford five subfractions (B651–B655). Subfraction B652 (45.7 mg) was purified by CC over Sephadex LH-20 using a mixture of methanol and dichloromethane in the ratio of 1:1 to afford three subfractions. The second subfraction (35.4 mg) was further separated by flash CC over silica gel with 30% acetone in dichloromethane as a mobile phase to afford two subfractions. The second subfraction (15.4 mg) was separated by dissolving with chloroform to afford a chloroform-insoluble fraction, which contained **5** (3.6 mg). Subfraction B654 (11.8 mg) was purified using the same procedure as subfraction B652 to give **13** (5.5 mg). Fraction C (110.8 mg) was purified by CC over reverse phase C_{18} silica gel using a gradient of methanol in water to give eight subfractions (C1–C8). Subfraction C2 (12.2 mg) was purified using the same procedure as subfraction B53 to give **8** (3.3 mg). Subfraction C4 was purified by CC over silica gel with a gradient of methanol in dichloromethane to yield six subfractions (C41–C46). Subfractions C41 and C45 contained **14** (1.7 mg) and **1** (3.5 mg), respectively. The third subfraction (13.9 mg) was purified by CC over reverse phase C_{18} silica gel with 30% acetone in water to give **15** (4.9 mg) and **16** (7.7 mg). Subfraction C7 contained **17** (6.2 mg).

*4.3.1. Xylariacyclone A (**1**).* Colorless gum; $[\alpha]_D^{25}$ +42.3 (*c* 0.39, EtOH); UV (MeOH) λ_{max} (log ε) 221 (3.74), 248 (3.71), 287 (3.32) nm; IR (neat) ν_{max} 3394, 1668 cm^{-1}; ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data ($CDCl_3$+CD_3OD), see Table 1; HREIMS *m*/*z* $[M]^+$ 340.1166 (calcd for $C_{16}H_{20}O_8$, 340.1153).

*4.3.2. Xylariacyclone B (**2**).* Colorless gum; $[\alpha]_D^{25}$ +23.6 (*c* 0.39, EtOH); UV (MeOH) λ_{max} (log ε) 247 (3.38), 298 (2.49), 345 (2.40) nm; IR (neat) ν_{max} 3402, 1737, 1667 cm^{-1}; ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data ($CDCl_3$), see Table 1; HRESIMS *m*/*z* $[M+Na]^+$ 188.0676 (calcd for $C_8H_{12}O_5Na$, 188.0679).

*4.3.3. Xylarinonericin A (**3**).* Colorless gum; $[\alpha]_D^{25}$ −32.5 (*c* 0.39, EtOH); UV (MeOH) λ_{max} (log ε) 247 (3.90), 266 (3.36), 330 (2.56) nm; IR (neat) ν_{max} 3401, 1712, 1674 cm^{-1}; ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data ($CDCl_3$), see Table 2; HRESIMS *m*/*z* $[M+Na]^+$ 525.2459 (calcd for $C_{28}H_{38}O_8Na$, 525.2464).

*4.3.4. Xylarinonericin B (**4**).* Colorless gum; $[\alpha]_D^{25}$ −18.6 (*c* 0.39, EtOH); UV (MeOH) λ_{max} (log ε) 245 (3.67), 289 (3.42), 349 (3.26) nm; IR (neat) ν_{max} 3409, 1729, 1694, 1660 cm^{-1}; ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data ($CDCl_3$), see Table 2; HRESIMS *m*/*z* $[M+Na]^+$ 697.2838 (calcd for $C_{35}H_{46}O_{13}Na$, 697.2836).

*4.3.5. Xylarinonericin C (**5**).* White solid; mp 147.5–149.0 °C; $[\alpha]_D^{25}$ −28.9 (*c* 0.39, EtOH); UV (MeOH) λ_{max} (log ε) 247 (4.07), 291 (3.13), 331 (2.89) nm; IR (neat) ν_{max} 3409, 1724, 1694, 1668 cm^{-1}; ^{1}H NMR

(300 MHz) and ^{13}C NMR (75 MHz) data (CD_3OD), see Table 2; HRESIMS m/z $[M+Na]^+$ 715.2937 (calcd for $C_{35}H_{48}O_{14}Na$, 715.2942).

*4.3.6. Xylariamide (**6**).* Colorless gum; $[\alpha]_D^{26}$ +26.6 (*c* 1.00, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 218 (3.07) nm; IR (neat) ν_{max} 3313, 1644 cm^{-1}; ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) data ($CDCl_3$), see Table 3; HREIMS m/z $[M+H]^+$ 188.1644 (calcd for $C_{10}H_{22}NO_2$, 188.1645).

*4.3.7. Preparation of the acetonide derivative of compound **1** (**1a**).* To a solution of **1** (1.1 mg) in 2,2-dimethoxypropane (0.6 mL) was added *p*-toluenesulfonic acid (1.0 mg) and the mixture stirred at room temperature for 5 h. Saturated aq $NaHCO_3$ was added, and the reaction mixture was extracted with EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to yield a reaction mixture (1.8 mg). The mixture was purified by PTLC using 100% CH_2Cl_2 (3 runs) to give **1a** (0.8 mg, 65% yield) as a colorless gum.

*4.3.8. Hydrolysis of compound **5**.* To a solution of **5** (1.5 mg) in a mixture of THF (40 μL) and H_2O (40 μL) was added LiOH (1.1 mg) and the reaction mixture stirred at room temperature for 2 h. A solution of 1 M HCl (200 μL) was added, and the reaction mixture was extracted with EtOAc. The combined EtOAc layers were evaporated to dryness under reduced pressure to provide sordaricin (0.8 mg) of which its optical rotation, $[\alpha]_D^{27}$ −79.1 (*c* 0.10, MeOH), was similar to the reported data for sordaricin, $[\alpha]_D^{20}$ −58.4 (*c* 0.19, MeOH).[24] The ^1H NMR spectroscopic data and TLC chromatogram were identical to those of the authentic sordaricin.

4.4. Antifungal assay

The pure compounds were tested for antifungal activity against *C. albicans* and *C. neoformans* at the concentration of 200 μg/mL using a colorimetric broth microdilution test.[42,43] A stock solution (10 mg/mL) was diluted with Roswell Park Memorial Institute (RPMI-1640) medium to 400 μg/mL and 50 μL of each test solution was pipetted into three wells of a 96 well plate. Each inoculum of 50 μL was added to the test solution and incubated at 35 °C for 15 h (*C. albicans*) and 25 °C for 45 h (*C. neoformans*). Then, 10 μL of 0.18% resazurin was added into each well and further incubated for another 2–3 h for *C. albicans* and *C. neoformans*. Amphotericin B was used as positive control. The color change was then observed visually. A blue or purple color of the wells indicated inhibition of growth (positive result). Any color changes from purple to pink or colorless were recorded as negative result. Amphotericin B exhibited the MIC value of 0.063 μg/mL.

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