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Guaiane sesquiterpenes and isopimarane diterpenes from an endophytic fungus *Xylaria* sp.

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ABSTRACT

Nine oxygenated guaiane-type sesquiterpenes and three isopimarane diterpenes were isolated from the culture broth of an endophytic fungus, *Xylaria* sp. YM 311647, obtained from *Azadirachta indica*. The structures of these compounds were elucidated by interpretation of spectroscopic data. The absolute configurations of two of these were confirmed by X-ray crystallographic analysis. All of the compounds were tested for their antifungal activities against five pathogenic fungal cells. The results showed that nine sesquiterpenes were moderately active against *Candida albicans* and *Hormodendrum compactum* with MIC values ranging from 32 to 256 μg/ml, while the diterpenes were more active; One of these exhibited the most potent inhibitory activity against *C. albicans* and *Pyricularia oryzae* with MIC values of 16 μg/ml.

1. Introduction

Endophytic fungi are often found living in apparently healthy plants and cause no apparent symptoms of disease for the host plant (König et al., 1999). They have proven to be the source of a wide range of novel and bioactive secondary metabolites (Gunatilaka, 2006; Schulz et al., 2002; Zhang et al., 2006). Previous work on the bioactive secondary metabolites from endophytic fungi residing in *Azadirachta indica* led to isolation of ten-membered lactones from *Phomopsis* sp. (Wu et al., 2008) and solanapyrone analogues from *Nigrospora* sp. (Wu et al., 2009). In a continual effort to search for new bioactive compounds from endophytic fungi in *A. indica*, nine new oxygenated guaiane-type sesquiterpenes (**1**–**9**) (Fig. 1) and three new isopimarane diterpenes (**10**–**12**) were identified from the culture broth of an endophytic fungus, *Xylaria* sp. YM 311647, isolated from this plant. Herein described are the isolation, structural elucidation, and *in vitro* antifungal activities of these new compounds.

2. Results and discussion

Compounds **1**–**7** were determined to have the identical molecular formula of $C_{15}H_{28}O_4$ on the basis of their HRESIMS data. The ^1H and ^{13}C NMR spectra indicated that all are tetrahydroxy derivatives of guaiane-type sesquiterpene, with the same distribution of methyl groups and one oxygenated methylene. In addition, compounds **1**–**4** contain five methylenes, three methines, and three oxygenated quaternary carbons, while compounds **5**–**7** contain four methylenes, five methines (one oxygenated), and two oxygenated quaternary carbons.

Compound **1** was obtained as colorless crystals from the $CHCl_3$–MeOH solution. The IR spectrum exhibited a typical absorption band at 3421 cm^{-1} accounting for OH groups. The ^{13}C NMR spectrum (Table 1) indicated the presence of 15 carbons all sp^3-hybridized, and these data, together with two degrees of unsaturation deduced from the molecular formula, suggested a bicyclic structure for **1**. The ^1H NMR spectrum (Table 2) showed that one of the methyl groups at δ 1.04 was a doublet and the other two at δ 1.27 and 1.41, respectively, were single attached to quaternary centers. Detailed analysis of the ^1H–^1H COSY, HMQC, and HMBC spectra led to assignment of a guaiane ring system. The coupling sequence from H-1 to H-4 and from H-6 to H-9 could be established by tracking correlations in the ^1H–^1H COSY spectrum. The ^1H and ^{13}C NMR spectroscopic data of **1** showed similarities to

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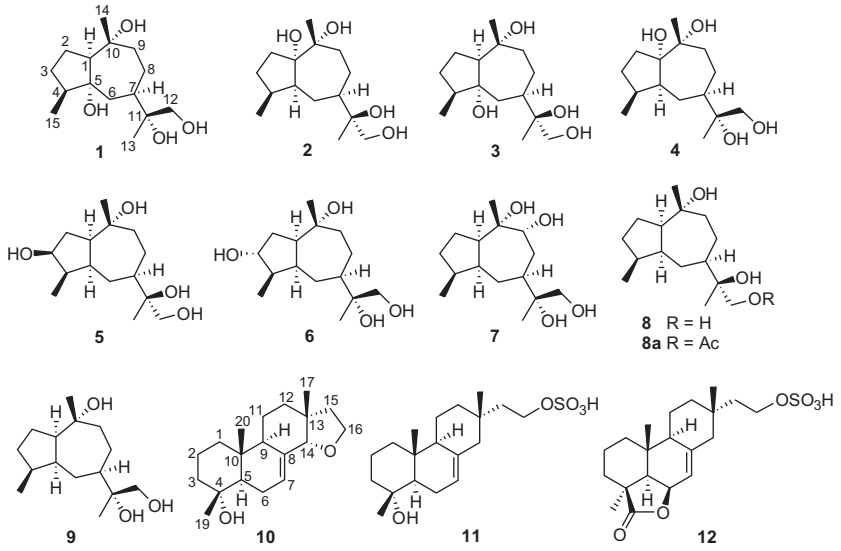

Fig. 1. Structures of compounds **1**–**12**.

Table 1
^{13}C NMR spectroscopic data of compounds **1**–**9** and **8a** (pyridine-d_5, 125 MHz, δ in ppm).

Position	1	2	3	4	5	6	7	8	8a	9
1	61.3	89.4	61.3	89.5	54.2	54.5	51.8	58.0	57.1	57.2
2	24.8	35.3	24.9	35.3	38.2	38.2	27.7	28.6	27.3	27.4
3	29.2	31.7	29.2	31.8	78.6	78.7	33.2	33.3	32.0	32.2
4	47.7	37.9	47.6	37.9	49.5	49.6	40.2	41.5	40.3	40.4
5	82.5	57.1	82.5	57.2	47.2	47.5	46.9	49.3	48.4	48.3
6	28.7	25.6	30.3	24.4	26.6	25.1	26.6	25.7	23.9	23.3
7	37.6	45.9	38.0	45.5	46.9	46.4	42.3	47.0	46.2	45.6
8	25.6	25.4	25.0	26.1	25.9	26.5	36.5	26.3	25.1	26.0
9	33.8	35.5	34.4	35.3	37.9	36.9	75.8	37.1	34.9	35.4
10	75.9	77.0	75.9	76.9	74.3	74.2	77.9	75.6	74.3	74.4
11	76.5	76.4	76.6	76.5	76.4	76.5	76.2	77.7	75.0	76.6
12	70.0	69.3	69.8	69.9	69.4	69.9	69.9	70.4	71.2	69.9
13	21.2	22.8	22.7	20.7	22.7	20.8	21.2	23.8	23.0	20.7
14	33.5	28.7	33.2	28.8	31.4	31.8	27.1	33.4	32.9	32.7
15	14.8	17.2	14.8	17.4	15.5	15.6	17.2	18.5	17.4	17.5
12-OAc									171.8	
									21.7	

Table 2
^{1}H NMR spectroscopic data of compounds **1**–**5** (pyridine-d_5, 500 MHz, δ in ppm, J in Hz).

Position	1	2	3	4	5
1	2.39 br t (10.2)		2.39 br t (10.1)		2.88 dd (17.6, 9.6)
2α	1.46 overlap	1.80 m	1.44 overlap	1.81 m	2.13 m
2β	1.76 m	2.05 overlap	1.75 m	2.04 m	2.26 m
3α	1.80 m	1.97 overlap	1.81 m	2.02 m	4.21 m
3β	1.19 ddd (22.3, 12.0, 5.2)	1.28 m	1.18 ddd (22.6, 11.8, 4.9)	1.29 m	
4	2.28 m	2.79 m	2.25 m	2.85 m	2.30 m
5		2.19 m		2.22 m	2.66 m
6α	2.47 br d (13.9)	1.97 overlap	2.14 br d (14.1)	2.32 br d (13.4)	2.04 br d (13.5)
6β	1.46 overlap	1.13 m	1.46 overlap	1.15 br dd (23.7, 12.9)	1.27 br dd (23.2, 12.9)
7	3.13 dd (17.9, 10.3)	2.52 m	3.12 dd (17.3, 10.3)	2.59 m	2.45 m
8α	2.32 m	2.46 m	2.51 m	2.29 overlap	2.48 m
8β	1.57 m	1.72 m	1.89 m	1.44 m	1.79 m
9α	2.02 m	2.05 overlap	2.04 m	2.29 overlap	2.13 m
9β	1.68 m	1.99 m	1.77 m	1.98 m	1.77 m
12a	3.99 d (10.6)	3.96 d (10.6)	4.06 d (10.8)	3.95 d (10.6)	4.01 d (10.7)
12b	3.90 d (10.6)	3.91 d (10.6)	4.02 d (10.8)	3.86 d (10.6)	3.95 d (10.7)
13	1.41 s	1.45 s	1.52 s	1.36 s	1.48 s
14	1.27 s	1.47 s	1.28 s	1.50 s	1.36 s
15	1.04 d (6.9)	0.94 d (7.1)	1.02 d (7.0)	1.01 d (7.1)	1.14 d (7.0)

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