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TWO NEW ACORANE-TYPE SESQUITERPENOIDS FROM *Aschersonia* sp. D20, AN ENDOPHYTIC FUNGUS OF *Annona squamosa*

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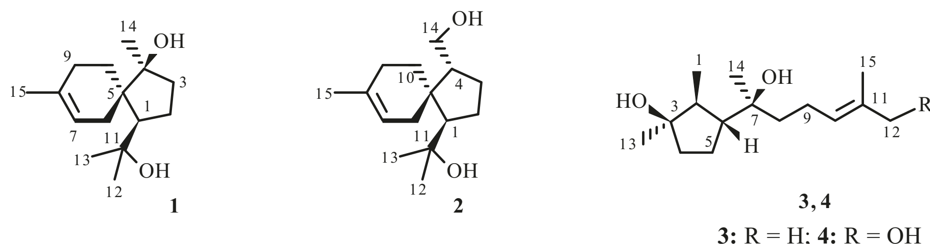
*Two new acorane-type sesquiterpenoids, 4-hydroxy-α-acorenol (**1**) and 14-hydroxy-α-acorenol (**2**), together with cyclonerodiol (**3**) and cyclonerotriol (**4**), were isolated from the culture extract of* Aschersonia *sp. D20, an endophytic fungus of* Annona squamosa*. Their structures were elucidated on the basis of IR, MS, and 1D and 2D NMR analyses.*

Keywords: acorane-type sesqiuterpenoid, *Aschersonia* sp. D20.

Plant endophytes are important sources of bioactive compounds, presumably due to the symbiotic relationship with their hosts [1–4]. Many members of the family Annonaceae are used in folk medicine for antiparasitic or antitumoral treatment of intestinal diseases [5]. *Annona squamosa* L. produces several types of *Annonaceous acetogenins* that showed cytotoxicity against several human cancer cell lines [6]. In such a special living environment inside *A. squamosa*, special endophytic fungi may exist and play an important role in producing bioactive compounds. Due to long-term coevolution, endophytes from this plant may possess abundant and novel secondary metabolites [7–9].

In recent years, many new and bioactive compounds have been isolated from the endophytic fungi of *A. squamosa* [10–13]. In order to search for more new skeleton and bioactive compounds, *Aschersonia* sp. D20, an endophytic fungal strain of *A. squamosa*, was selected. Here, we report the isolation and structure elucidation of two new acorane-type sesquiterpenoids **1** and **2** from *Aschersonia* sp. D20.



Compound **1** was obtained as colorless crystals, $[\alpha]_D^{20}$+38° (*c* 0.1, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 227 (2.42), 281 (0.70). The ^{1}H NMR (Table 1) indicated the presence of one olefinic proton (δ 5.40 m) and four tertiary methyls (δ 1.06 s, 1.17 s, 1.18 s, and 1.64 s). The ^{13}C NMR (Table 1) spectra of **1** exhibited 15 carbons, which were assigned by HMQC, HMBC, and DEPT experiments to the resonances of four methyls, five methylenes, two methines (one olefinic), and four quaternary C atoms (one olefinic and two oxygenated). The IR spectrum showed hydroxyl groups (3306 cm^{-1}) and a carbon-carbon double bond (1643 cm^{-1}), and its molecular formula was established as $C_{15}H_{26}O_2$ by HR-ESI-MS ([M + Na]$^+$, *m/z* 261.2067), indicating a bicyclic sesquiterpenoid. Detailed 2D NMR analysis showed that compound **1** is an acorane-type sesquiterpene [14].

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TABLE 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data for Compounds **1** and **2** (acetone-d_6, δ, ppm)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	1.82 (m)	54.4 d	1.81 (m)	55.6 d
2	1.78 (m), 1.93 (m)	24.1 t	1.85 (2H, m)	24.7 t
3	1.54 (m), 1.73 (m)	37.7 t	1.69 (2H, m)	24.4 t
4	–	82.5 s	1.98 (m)	48.8 d
5	–	48.6 s	–	45.6 s
6	2.01 (m), 2.53 (m)	26.4 t	1.88 (m), 2.60 (m)	31.3 t
7	5.40 (m)	123.0 d	5.32 (m)	121.1 d
8	–	132.3 s	–	132.5 s
9	1.83 (m), 2.01 (m)	26.7 t	1.91 (2H, m)	28.5 t
10	1.22 (m), 1.31 (m)	32.4 t	1.48 (m), 1.94 (m)	30.2 t
11	–	71.8 s	–	72.1 s
12	1.17 (3H, s)	31.2 q	1.18 (3H, s)	30.4 q
13	1.18 (3H, s)	31.2 q	1.23 (3H, s)	30.5 q
14	1.06 (3H, s)	20.5 q	3.36 (m), 3.71 (m)	62.2 t
15	1.64 (3H, s)	22.6 q	1.61 (3H, s)	22.6 q

The oxyisopropyl group was assigned by the HMBC correlations from H-12 and H-13 to C-11 (δ 71.8) and C-1 (δ 54.4), the correlations from H-1 to C-12 (δ 31.2) and C-13 (δ 31.2), and the low chemical shift of C-11 at δ 71.8. The H-2 correlations with carbons C-11 and C-1, the H-14 correlations with carbons C-3 (δ 37.7), C-4 (δ 82.5), and C-5 (δ 48.6), and the ^{1}H–^{1}H COSY connections between H-2 and H-3 provided the fragment 3-(2-hydroxypropan-2-yl)-1-methylcyclopentanol. Similarly, the H-15 correlations with carbons C-7 (δ 123.0), C-8 (δ 132.3), and C-9 (δ 26.7) and the ^{1}H–^{1}H COSY also showed connections between H-7 and H-6, and between H-9 and H-10. These data showed similarities to those of α-acorenol [15, 16], except for the absence of the CH group with a signal at δ 41.8, and the presence of an additional *O*-bearing carbon group with a signal at δ 82.5. Therefore, compound **1** was elucidated as 4-hydroxy-α-acorenol.

The relative configuration of **1** was established by the NOE correlations between H-6 and H-12 and H-13, and H-1 and H-6.

Compound **2**, white powder, $[\alpha]_D^{20}$ –57° (*c* 0.1; MeOH). UV (MeOH, λ_{max}, nm) (log ε): 227 (2.42), 281 (0.70). IR (KBr, ν_{max}, cm^{-1}): 3306, 2964, 2933, 1643, 1530. The ^{1}H NMR of **2** (Table 1)indicated the presence of one olefinic proton (δ 5.32 m), three tertiary methyls (δ 1.23 s, 1.18 s and 1.61 s), and one oxymethylene group (δ 3.36 m and 3.71 m). The ^{13}C NMR and DEPT spectra of **1** (Table 1) exhibited 15 resonances ascribed to three methyls, six methylenes, three methines (one olefinic), and three quaternary C-atoms (one olefinic and one oxygenated). The NMR spectrum suggested that compound **2** has the same skeleton as α-acorenol. The difference between them is that the methyl at C-14 was oxygenated. Compound **2** was elucidated as 14-hydroxy-α-acorenol. The structure was further corroborated by the HR-ESI-MS at *m/z* 261.2055 $[M + Na]^+$ and HMBC and ^{1}H–^{1}H COSY correlations. The relative configuration of **2** was proposed to be the same as that of **1** according to the NOE correlations.

The two known compounds, cyclonerodiol **3** [17, 18] and cyclonerotriol **4** [19], were also isolated and identified by comparing their spectral data with the literature. Interestingly, the sesquiterpenes isolated from the endophytic fungus *Aschersonia* sp. D20 belong to two structural types, acorane (**1** and **2**) and cyclonerane (**3** and **4**), which reveals the diversity of its terpenoid constituents.

EXPERIMENTAL

General Experimental Procedures. Precoated silica gel GF_{254} plates for TLC and column chromatography (CC) silica gel (Qingdao Marine Chemical Factory, Qingdao, P. R. China), RP-18 gel (40–63 μm, Merck) and Sephadex LH-20 gel (Amersham Biosciences). IR spectrum: Thermo Nicolet 380 FT-IR spectrophotometer, with KBr pellets, in cm^{-1}. NMR spectra: Bruker ARX 600 spectrometer operating at 600/150 MHz, δ in ppm relative to Me_4Si; J in Hz. HR-ESI-MS: Bio TOF^{TM} Q mass spectrometer (Bruker), in *m/z*.

Isolation and Identification of the Endophytic Fungus. The fungus was isolated from surface-sterilized fresh phloems of an apparently healthy *Annona squamosa* L. collected in Xiamen University, Xiamen, Fujian Province, P. R. China.

The fungal strain *Aschersonia* sp. D20 was isolated by repeated subculturing and deposited in Xiamen University. Both traditional morphology and internal transcribed spacer (ITS) sequencing were performed to characterize it as *Aschersonia* sp. Fermentation was performed for 14 days at 28°C on potato-dextrose-agar (PDA) media (4 L).

Extraction and Isolation. The cultured agar was chopped, diced, and extracted overnight with EtOAc–MeOH–AcOH 80:15:5 three times. The organic solution was collected by filtration. The combined filtrates were concentrated to remove the organic solvents. The aqueous solution was extracted with EtOAc. The combined organic EtOAc layer, upon solvent removal, yielded the EtOAc extract (1.4 g). The EtOAc extract (4.7 g) was subjected to MPLC [RP-18 silica gel (80 g), 30, 50, 70, and 100% acetone–H_2O, 1 L for each gradient step] to yield fractions 1–7. Fraction 4 (20 mg) was separated by CC [Sephadex LH-20 (140 g), MeOH] to yield Fr. 4a. Fraction 4a (6 mg) was further purified by CC (SiO_2, petroleum ether–$CHCl_3$, 5:8) to yield **1** (2 mg). Fraction 3 (110 mg) was separated by CC [Sephadex LH-20 (140 g), MeOH] to yield Fr. 3a (20 mg) and 3b (60 mg). Fraction 3a was then subjected to Sephadex LH-20 column chromatography (70 g, acetone) to yield Fr. 3a1 (6 mg). Fraction 3a1 was further purified by CC (SiO_2, petroleum ether–$CHCl_3$, 1:4) to yield **3** (4 mg). Fraction 3b was subjected to MPLC [RP-18 silica gel (30 g), eluted with 35% acetone] to yield Fr. 3b1 (20 mg). Fraction 3b1 was finally purified by CC (SiO_2, petroleum ether–EtOAc, 10:1) to yield **2** (4 mg).

Fraction 2 (90 mg) was separated by CC [Sephadex LH-20 (140 g), MeOH] to yield Fr. 2a (7 mg). Fraction 2a was then subjected to Sephadex LH-20 column chromatography (70 g, acetone) to yield Fr. 2a1 (5 mg). Fraction 2a1 was further purified by CC (SiO_2, $CHCl_3$–MeOH, 80:1) to yield **4** (2 mg).

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