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α-Pyrone derivatives from the endolichenic fungus *Nectria* sp.

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ABSTRACT

Five new α-pyrone derivatives, necpyrones A–E (**1–5**), together with seven known compounds (**6–12**), were isolated from the extract of an endolichenic fungus *Nectria* sp. Chemical structures of these compounds were elucidated by spectroscopic analyses (HRESIMS and NMR). The absolute configurations of C-6 in the pyrone ring as well as the chiral carbons at the aliphatic side chain resulted from hydroxyl substitute were finally determined on the basis of measurement of ECD and Kusumi–Mosher method.

1. Introduction

α-Pyrone derivatives were found to be distributed in variety of fungi, *Penicillium* sp., *Pestalotiopsis photiniae*, *Pestalotiopsis virgatula*, *Phomopsis amygdali*, and so on (McGahren et al., 1973; Ding et al., 2012; Rönsberg et al., 2013). This class of natural products showed an abroad spectrum of biological activities, such as gibberellin-synergistic, antimicrobial, spasmolytic, and cytotoxic effects (Kirihata et al., 1996; Luo et al., 2012; Zhang et al., 2013; Costa et al., 2014). Our ongoing search for new bioactive natural products from endolichenic fungi (Li et al., 2012) led to the isolation of five new α-pyrone derivatives necpyrones A–E (**1–5**) and seven known compounds (**6–12**) from the fungus *Nectria* sp., occurring in the lichen *Pamelia* sp., collected from Zhejiang Province, China. Details of the isolation, structure elucidation, and cytotoxicity elucidation of these metabolites are reported herein (Fig. 1).

2. Results and discussion

Necpyrone A (**1**) was obtained as a colorless oil. Its molecular formula was determined as $C_{11}H_{18}O_5$ by HRESIMS at *m*/*z* 231.1227 $[M+H]^+$, corresponding to three indices of hydrogen deficiency. Analysis of the ^1H, ^{13}C, and HSQC NMR data of **1** (Table 1) revealed the presence of an olefinic bond (δ_C 173.5, C-4; δ_C 90.0, C-3; δ_H 5.12, H-3), a methyl (δ_C 23.6, C-5′; δ_H 1.20, H-5′), a methoxyl (δ_C 56.3, δ_H 3.74, 4-OMe), three methylenes (δ_C 29.5, C-5; δ_H 2.80, H-5α; δ_H 2.28, H-5β; δ_C 28.7, C-2′; δ_H 1.73, H-2′; δ_C 35.1, C-3′; δ_H 1.73, H-3′a; δ_H 1.59, H-3′b), three methines (δ_C 78.8, C-6; δ_H 4.33, H-6; δ_C 72.4, C-1′; δ_H 3.69, H-1′; δ_C 67.8, C-4′; δ_H 3.88, H-4′), and two quaternary carbons (δ_C 167.3, C-2; δ_C 173.5, C-4). The presence of the 1,4-diol moiety was established by the ^1H–^1H COSY cross peaks from H-1′ to H-5′ and H-6. The part of pyrone ring was deduced on the basis of ^1H–^1H COSY correlations between H-5 and H-6, and HMBC correlations from H-3 to C-4 and C-5, and from H-5 to C-3 and C-4 (Fig. 2). Therefore, the planar structure of **1** was constructed as an α-pyrone derivative, closely related to those of (6*S*, 1′*S*)-LL-P880α (**7**) (Akay et al., 2014), except for the presence of an additional hydroxyl group at C-4′ (δ_C 67.8) in **1**.

The absolute configuration of C-6 was determined as 6*S* on the basis of the negative Cotton effect at 249 nm ($\Delta\varepsilon$ −9.96) in ECD spectrum (Fig. 3a) (McGahren et al., 1973). The absolute configuration of the 1′,4′-diol moiety in **1** was established by Kusumi–Mosher method (Seco et al., 2004; Li et al., 2014). The differences of the ^1H NMR chemical shift values between (*R*)- and (*S*)-MTPA ester derivatives (**1a** and **1b**) ($\Delta\delta = \delta_S - \delta_R$) were calculated to assign the 1′*S* and 4′*S* configurations by applying the 1,4-anti-diol model as reported by Riguera et al. (Fig. 4).

Necpyrone B (**2**) was also isolated as a colorless oil. The same molecular formula as that of **1**, $C_{11}H_{18}O_5$, was determined by HRESIMS (*m*/*z* 231.1228 $[M+H]^+$). The spectroscopic data of compound **2** (Table 1) was highly similar to that of **1** except for the delicate differences assigned to the aliphatic side chain (C-2′–C-3′–C-4′–C-5′). Meanwhile, the similar ECD spectra (Fig. 3a) suggested that compound **2** had the same absolute configurations of 6*S* and 1′*S* as those of **1**. We derivatized **2** as the strategy to **1** to determine the configuration of the 1′,4′-diol moiety by the Kusumi–Mosher method. Analysis of the ^1H NMR chemical shift

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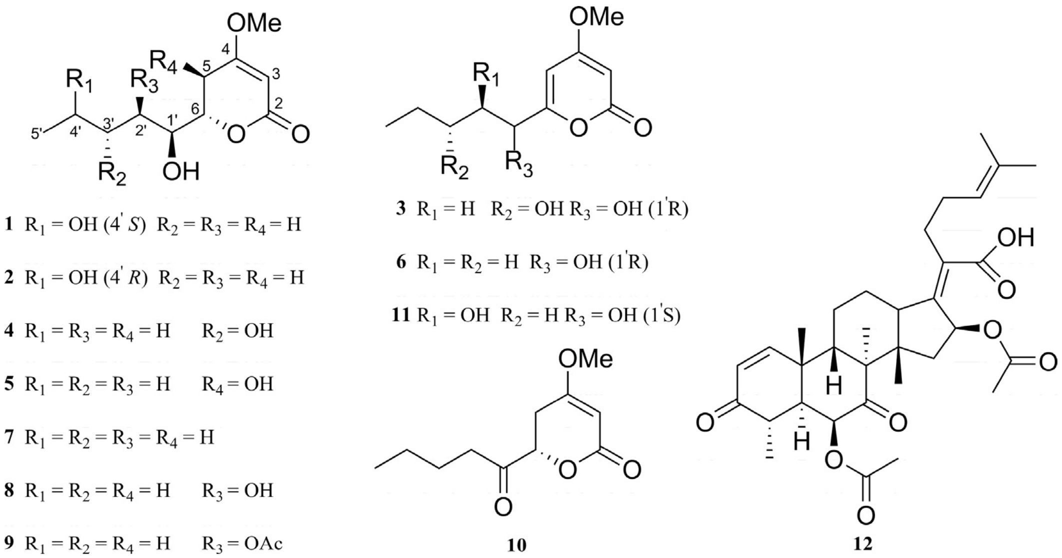

Fig. 1. Structures of compounds **1**–**12**.

Table 1
NMR data for compounds **1** and **2**.

Position	**1**		**2**	
	δ_C mult.[a]	δ_H mult.[b] (J in Hz)	δ_C mult.[a]	δ_H mult.[b] (J in Hz)
2	167.3, C		167.0, C	
3	90.0, CH	5.12 d (1.7)	90.1, CH	5.14 d (1.3)
4	173.5, C		173.4, C	
5α	29.5, CH_2	2.80 ddd (1.7, 4.2, 12.9)	29.5, CH_2	2.81 ddd (1.3, 4.1, 12.9)
5β		2.28 dd (3.7, 17.1)		2.29 dd (3.7, 17.1)
6	78.8, CH	4.33 dt (4.2, 12.9)	78.7, CH	4.34 dt (4.1, 13.0)
1′	72.4, CH	3.69 m	72.5, CH	3.71 m
2′	28.7, CH_2	1.73 m[c]	29.4, CH_2	1.75 m
3′a	35.1, CH_2	1.73 m[c]	35.4, CH_2	1.69 m
3′b		1.59 m		1.63 m
4′	67.8, CH	3.88 m	68.2, CH	3.89 m
5′	23.6, CH_3	1.20 d (6.2)	24.0, CH_3	1.23 d (6.2)
4-OMe	56.3, CH_3	3.74 s	56.3, CH_3	3.76 s

[a] Recorded in $CDCl_3$ at 150 MHz; ^{13}C multiplicities were determined by HSQC experiment.
[b] Recorded in $CDCl_3$ at 600 MHz.
[c] Signals overlapped.

differences ($\Delta\delta = \delta_S - \delta_R$) between the (*S*)- and (*R*)-MTPA ester derivatives (**2b** and **2a**) led to the assignment of the 1′*S* and 4′*R* configuration at C-1′ and C-4′ (Fig. 4), respectively.

Necpyrone C (**3**), giving the molecular formula of $C_{11}H_{16}O_5$ by HRESIMS (*m*/*z* 229.1070 $[M+H]^+$), was obtained as a colorless oil. The ^1H and ^{13}C NMR data of **3** (Table 2) resembled those of PC-2 (**6**) (Rahbaek et al., 2003), except for the presence of an additional hydroxyl group at C-3′ in **3**, which was confirmed by the chemical shift of C-3′ (74.7) and ^1H–^1H COSY correlations of H-3′ with H-2′ and H-4′. The same Kusumi–Mosher method as that used for compounds **1** and **2** was applied to determine the absolute configurations of C-1′ and C-3′. Analysis of the ^1H NMR chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*S*)- and (*R*)-MTPA ester derivatives (**3b** and **3a**) led to the assignment of the *R*-configuration at both C-1′ and C-3′ (Fig. 4). The opposite Cotton effect at 282 nm (Δε +1.54) in the ECD spectrum of **3**, compared to the ECD spectrum of (1′*S*, 2′*R*)-LL-P880γ (**11**) (Fig. 3b), also supported the 1′*R* configuration.

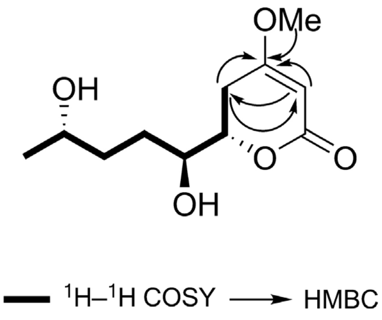

Fig. 2. Key HMBC and ^1H–^1H COSY correlations of **1**.

Necpyrone D (**4**), a colorless amorphous powder, has a molecular formula $C_{11}H_{18}O_5$, as determined by the $[M+H]^+$ at *m*/*z* 231.1228 in the HRESIMS. The chemical shift differences at C-1′ (69.7 vs. 73.4), C-2′ (38.9 vs. 30.8), and C-3′ (70.7 vs. 72.8) with those of photipyrone C (Ding et al., 2012), the stereoisomer of **4**, indicated the different configurations of these two compounds. The same absolute configurations of 1′*S* and 6*S* in **4** as those in compound **1** were determined by the same positive Cotton effect at 219 nm and the negative one at 249 nm in ECD (Fig. 3a). The stereochemistry of 1′*S* and 3′*R* was further deduced by application of the Kusumi–Mosher method as that used above.

Necpyrone E (**5**), with the molecular formula $C_{11}H_{18}O_5$ from HRESIMS (*m*/*z* 231.1228 $[M+H]^+$), was obtained as a colorless amorphous powder. The ^1H and ^{13}C NMR data (Table 3) of **5** resembled those of photipyrone B (Ding et al., 2012), except for the chemical shift difference of C-5 (63.1 vs. 67.1) which implied the